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**RESEARCH ARTICLE**

# Paediatric Drug Development: Regulatory Framework as per USFDA

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## ABSTRACT

In the intricate world of paediatric drug development, the US Food and Drug Administration (USFDA) play a pivotal role, ensuring that medications are both safe and effective for children.This abstract explores the USFDA's regulatory system, emphasizing important policies and programs intended to close the gap between adult and pediatric pharmacotherapy. Legislative measures that require stringent clinical trials and provide incentives for research in pediatric populations, such the Pediatric Research Equity Act (PREA) and the Best Pharmaceuticals for Children Act (BPCA), are essential to this framework. The USFDA's strategy places a strong emphasis on age-appropriate formulations, moral issues, and how crucial it is to adjust dosages in accordance with pediatric physiology. Pharmaceutical businesses are better able to meet the special therapeutic needs of children by navigating the complicated regulatory environment, which eventually leads to advances that enhance paediatric health outcomes. The study provides significant new data on regulatory approach for those involved in the development of pediatric medicine.

**Keywords:** Paediatric, Drug development, Regulatory approach, USFDA, PREA, BPCA.

## INTRODUCTION

The process **of transforming a molecule from** a drug candidate (the end-product of the discovery phase) into a product that has been given the green light for commercialization by the relevant regulatory authorities is known as drug development (1). The complexity in drug development has increased manifolds over the past 40 years, requiring preclinical phase of drug development, investigational new drug (IND) application, and complete clinical

testing before marketing approval from the FDA. Generally, new drug applications (NDAs) or biologics license applications (BLA) are reviewed comprehensively before approval, and then drug performance is resubmitted to regulatory agencies for post-marketing studies. The overarching goal is to bring more efficient and safer treatments to the patients as quickly as possible after a thorough medical evaluation (2).

The USFDA drug development process consists of five essential components, each of which contains numerous phases and stages. We will go over each step and phase of the drug development process to gain a comprehensive understanding of the whole thing. The stages involved in developing drugs are:

Step 1: Discovery and Development
Step 2: Preclinical Research
Step 3: Clinical Development
Step 4: FDA Review
Step 5: FDA Post-Market Safety Monitoring

Discovery and Development: In this phase, experts from academia and industry collaborate to find pharmacological compounds that affect a specific biological target linked to a disease.

Preclinical Research: The goal of preclinical testing is to gather vital information about the safety and efficacy of a medication candidate before it is tested on humans. A candidate's biological impact is frequently illustrated using both in vitro and in vivo models.

Clinical Development: The purpose of clinical trials is to offer particular scientific information about a medication under research. The clinical trial study protocol specifies the procedures to be followed and the manner in which the clinical trial must be conducted. In order to guarantee the safety of the human subjects taking part in the trials, it provides details about the main goals of the study, its design, and statistical considerations. Clinical trials consist of three distinct phases, namely I, II, and III, which vary in terms of time, participant count, and study objectives.

FDA Review: When the sponsor or organization has enough evidence of the drug's safety and efficacy to satisfy FDA regulations for marketing approval, they submit a New Drug Application (NDA) for evaluation. The application for review must also contain data from specific technical viewpoints, such as chemistry, pharmacology, medicine, bio-pharmaceutics, and statistics. The product may be commercialized in the US if the NDA is accepted.

FDA Post-Market Safety Monitoring: Post-market safety surveillance is the process of keeping an eye on a medication after it has been approved and placed on the market. It is designed to evaluate the long-term safety and efficacy of a drug, possible formulation problems in "the real world," and use for unapproved conditions, or "off-label."(1) (2)

Patients who are 21 years of age or younger at the time of diagnosis or treatment are classified as paediatric patients under the Federal Food, Drug, and Cosmetic Act (FD&C Act). The groupings of paediatric patients are determined by their developmental patterns(3) (4).Table 1 represents the classification of paediatric population in USFDA

**REGULATORY LEGISLATION IN USFDA**

In the USA, a well-established regulatory system permits the development of paediatric drugs. The introduction of legislation such as the Paediatric Research Equity Act (PREA) in the United States reflects a commitment to addressing the unique challenges associated with paediatric drug development[(6).]

**Regulatory Pathways in the US**

The regulatory pathways for paediatric drugs involve a series of steps and considerations to ensure the safety and efficacy of pharmaceutical products specifically for the paediatric population. Two important pieces of legislation in

the US are Paediatric Research Equity Act (PREA) and Best Pharmaceuticals for Children Act (BPCA). These legislations play a significant role in shaping the regulatory framework for paediatric drugs [7].

**BEST PHARMACEUTICALS FOR CHILDREN ACT (BPCA):**

The BPCA permits studies aimed at enhancing children's medication use safety and effectiveness. The goal of BPCA is to enhance children's drug use and dose safety and effectiveness. In 2002, BPCA was enacted. The U.S. Food and Drug Administration (FDA) Amendments Act of 2007, the FDA Safety and Innovation Act of 2012, and the FDA Reauthorization Act of 2017 all served to reauthorize it. The BPCA laws were most recently renewed in 2022(8).

**Regulatory procedure in BPCA**

The regulatory procedure in the Best Pharmaceuticals for the Children Act (BPCA) involves two ways:

(1) The sponsor submitting the Proposed Paediatric Study Request (PPSR) to USFDA and requests the FDA issue a Written Request (WR) as given in Figure 1.

(2) Figure 2 describes the FDA issues WR to sponsor.

**Conditions for exclusivity**

To be eligible for exclusivity, a study must meet certain requirements. Firstly, the FDA must receive a written request. Studies that have already been submitted to the Agency prior to the WR's issuance cannot be granted paediatric exclusivity or a WR.

All the deadlines and conditions mentioned in the WR ought to be fulfilled. The active moiety needs to retain its exclusivity or patent life. Sponsors of off-patent drugs have no financial motivation to carry out trials in paediatric patients (11).

**Paediatric Exclusivity**

Applicants may be granted paediatric exclusivity provided they meet the requirements of a WR. An extra half-year of exclusivity (6 months). All existing patents and marketing exclusivities for the drug moiety are attached with exclusivity. Positive paediatric studies or the approval of a new indication are not necessary for paediatric exclusivity (12).

**PAEDIATRIC RESEARCH EQUITY ACT (PREA)** :

The Paediatric Research Equity Act amends the federal Food, Drug, and Cosmetic Act to authorize the FDA to require paediatric studies of drugs or biologics when other approaches are insufficient to ensure

**PAEDIATRIC STUDY PLAN (PSP)**

Unless the drug is for an indication for which orphan designation has been granted, a sponsor who plans to submit a marketing application (or supplement to an application) for a new active ingredient, new indication, new dosage form, new dosing regimen, or new route of administration must submit an Initial Paediatric Study Plan (iPSP).

For any new application or supplement subject to PREA, the sponsor is required to file an iPSP, even if the FDA has previously granted waivers or deferrals under PREA for the same medicine (14).

**Submission Timelines for PSP**

A sponsor must submit an iPSP, if required under PREA, before the date on which the sponsor submits the required assessments or investigation and no later than either 60 calendar days after the date of the end-of-phase 2 meeting.

After the sponsor submits an iPSP, the FDA has 90 days to review the iPSP and provide a written response to the iPSP, or meet with the sponsor to discuss the iPSP, as appropriate. This review process includes consultation with FDA's internal Paediatric Review Committee (PeRC)(15).

The sponsor then has a second 90-day period during which it may review FDA comments and initiate any needed negotiations to discuss the iPSP. By the end of this second 90-day review period, the sponsor must submit an agreed iPSP. The FDA then has 30 days after receipt of the agreed iPSP to review and issue correspondence confirming agreement or issue correspondence stating disagreement. If the FDA does not agree, the iPSP is considered a non-agreed iPSP(16). The pathway of submission of paediatric study plan is given in Figure 4(15).

## CONCLUSION

The study titled "Paediatric Drug Development: Regulatory Framework as per USFDA "aimed to address the regulatory framework inherent in developing pharmaceuticals tailored for paediatric patients. The results of the study provide insights into a strategic and systematic regulatory approach, emphasizing the importance of a sequential framework to guide the intricate journey of paediatric drug development. The study underscores the collaborative engagement of regulatory authorities, industry stakeholders, healthcare professionals, and patient advocacy groups as pivotal for success. By providing a clear roadmap, the framework aims to streamline the regulatory landscape, fostering innovation, and expediting the availability of safe and effective medications for paediatric patients. Furthermore, the study delves into the regulatory legislation in the United States, highlighting key acts such as the Paediatric Research Equity Act (PREA) and the Best Pharmaceuticals for Children Act (BPCA). The regulatory pathways outlined in the study, with a focus on the USA, illustrate the meticulous steps and considerations required to ensure the safety and efficacy of pharmaceutical products for the paediatric population. The study also provides a detailed overview of the Paediatric Study Plan (PSP) and its submission timelines, reinforcing the commitment to a systematic approach in paediatric drug development. It contributes a valuable resource for understanding the intricacies of regulatory pathways and study plan for paediatric drugs in USA.

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**Table 1: Classification of Paediatric Population as per USFDA**[5]

| Paediatric population | Age |
|---|---|
| Neonates | 0 to 28 days |
| Infants | 29 days to less than 2 years |
| Children | 2 to less than 12 years |
| Adolescents | 12 to 21 years |

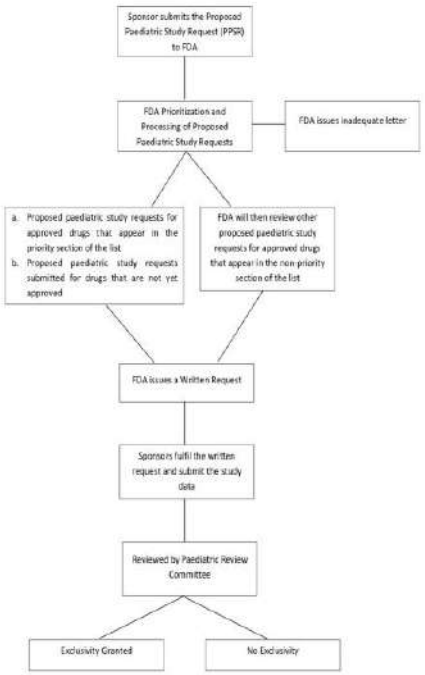


**Figure 1. Sponsor submits the Proposed Paediatric Study Request (PPSR) to FDA (09)**

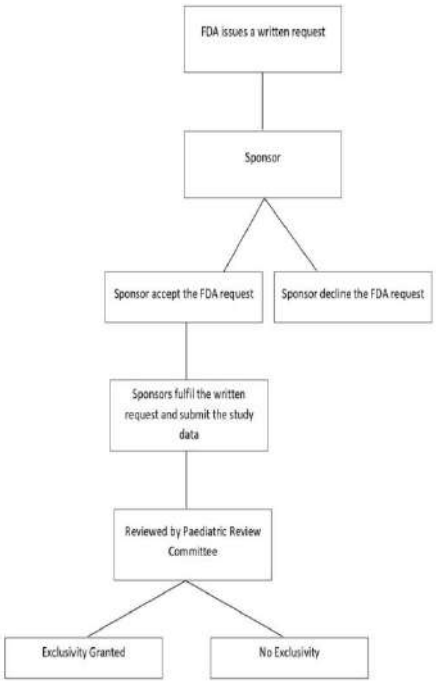


**Figure 2. FDA issues WR to sponsor** (10)

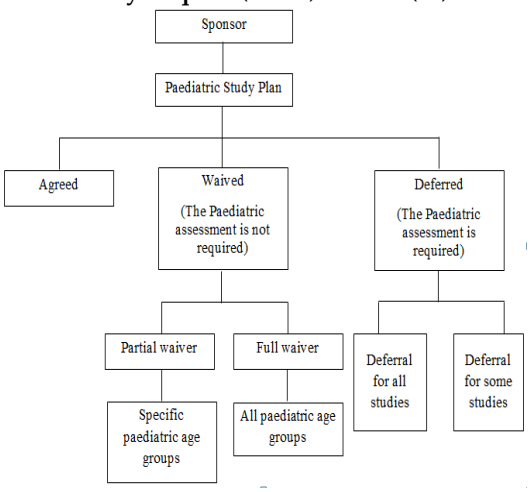


**Figure 3 Regulatory Pathway in PREA (13)**

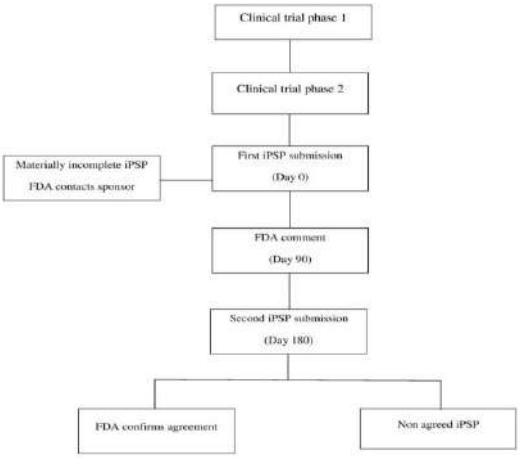


**Figure 4 Timeline of iPSP Submission** (15)

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**RESEARCH ARTICLE**

# Indexed Results and Tables Relating to the Quick Switching System (QSS-1) with a Single Sampling Plan as the Reference Plan

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**ABSTRACT**

This research examines the Quick Switching System (QSS-1) using a Single Sampling (SSP) Plan as a reference plan with various quality levels. The study provides performance measures and procedures for designing the system with different entry parameters. The main objective is to reduce inspection costs while maintaining an acceptable level of quality for the customer.

**Keywords:** Quick Switching System, Single Sampling Plan, Acceptable Quality Level, Limiting Quality Level, Palya Distribution.

## INTRODUCTION

QSS-SSP (n: $u_1$, $u_2$, $v_1$, $v_2$) refers to a quick switching system where the usual SSP plan has a sample of size 'n' and acceptance numbers $u_1$, $u_2$ ($u_1 < u_2$) and the tightened SSP plan has a sample of size n and acceptance numbers $v_1$, $v_2$ ($v_1 < v_2$, $v_1 \leq u_1$ and $v_2 < u_2$).

**Romboski (1969) observes the following advantages for QSS-1.**

- The composite OC curve of QSS-1 is having better shape (close to z shape) than the corresponding normal and tightened OC curves).
- As the tightening becomes plain the composite OC curve methods to the ideal form.
- The system results in reduction of sample size than the equivalent reference plan.

**Switching Procedures**

A sampling system encompasses of plans, which are acceptable to normal, tightened and reduced inspections. The switching rules are given below.

Normal to tightened:
When normal inspection is in effect tightened assessment shall be instituted when two out of five consecutive lots or batches have been rejected on original inspection.

Tightened to normal:
When tightened inspection is in effect normal inspection shall be instituted when five consecutive lots have been accepted on unique inspection.

Normal to reduced:
When normal inspection is in effect, reduced inspection shall be instituted providing that all the following conditions are fulfilled.

1. The previous 10 lots or batches have been on normal inspection and none has been on original inspection
2. The total number of uncharacteristic units in the sampling from the preceding 10 lots or batches is equal to or less than the applicable number
3. Production is at fixed rate and
4. Reduced inspection is considered desirably by the responsible authority.

Reduced to normal**:**

1. A lot or batch is rejected or
2. A lot or batch is measured acceptable under the process of MIL-STD-105D system procedures.
3. Production becomes uneven or delayed
4. Other conditions warrant that normal inspection shall be instituted.

This section gives a review on Quick Switching System-r (n, $c_N$, $c_T$) where r = 1, 2, 3 are discussed. The contributions made by the author are stated at the end of the sections.

Dodge (1967) proposed a new sampling system consisting of pairs of normal and tightened plans. The application of the system is as follows

1. Adopt a pair of sampling plans, a normal plan (N) and tightened plan (T), the plan T to be tightened OC curve wise than plan N.
2. Use plan N for the first lot (optional): can start with plan T; the OC curve properties are the same ; but first lot protection is greater if plan T is used.
3. For each lot inspected; if the lot is accepted, use plan N for the next lot and if the lot is rejected, use plan T for the next lot'.

Due to instantaneous switching between normal and tightened plan, this system is referred as " Quick Switching System ". Using the concept of Markov Chain, the OC function of QSS-1 is derived by Romboski (1969) as

$$Pa(p) = P_T / (1-P_N+P_T) \qquad (1)$$

Romboski(1969) introduced QSS-1 (n; $c_N$.$c_T$) which is a QSS-1 with single sampling plan as a mention plan [ (n, $c_N$) and (n, $c_T$) are respectively the normal and tightened single sampling plans with $c_T < c_N$ ].

**Designation of Quick Switching System**

Romboski (1969) has extensively studied QSS by taking pairs of single sampling plan. The designation of the system is as follows :

i) QSS(n;$c_N$,$c_T$) – refers to a QSS where the single sampling normal plan has a sample size of n and an acceptance number of $c_N$, and the tightened single sampling plan has the same sample size as that of the normal plan but with acceptance number $c_T$. In general, $c_T \leq c_N$ and when $c_T = c_N$ then the system degenerates into a single sampling plan.

ii) QSS(n,kn;$c_0$)) – refers to a QSS where the normal and tightened single sampling plans hae the same acceptance number but on tightened inspection the sample size is a multiple of k (k≥1) of the sample size on normal inspection. If k = 1, the system degenerates into single sampling plan.

Romboski (1969) has given the QC function of QSS (n; $c_N$, $c_T$) and QSS (n, kn; $c_0$) as

$$Pa(p)= \frac{P_T}{1-P_N+P_T}$$

Where $P_N$ and $P_T$ are explained.

**SINGLE SAMPLING PLAN**

A brief note on single sampling plan is given in this section. A single sampling plan is characterized by sample size n and the acceptance number c, sampling inspection in which the decision to accept or not to accept a lot is based on the inspection of a single sample of size n.

**Operating procedure**

Select a random sample of size n and count the number of non-conforming units d. If there is c or less non – conforming units, the lot is accepted, otherwise the lot is rejected. Thus the plan is characterized by two parameters viz, the sample size n and the acceptance number 'c'. The OC function of the single sampling plan is given as

$$P_a(p) = P(d < c, n) \qquad (2)$$

Peach and littauer (1946) have given tables for determining the single sampling plan for static $\alpha = \beta = 0.05$. they have used the relation that for even degrees of freedom $x^2$ gives the outline of a poisson distribution as the basis for developing tables of a single sampling plan. they have introduced the concept of the operating ratio $p_2/p_1$ as a measure for the power of discrimination of the OC curve. The values of $p_2/p_1$ and $np_1$ are intended against different values of c for fixed $\alpha = \beta = 0.05$ using the table, a single sampling plan can be selected for given $p_1$ and $p_2$.

Burgess (1948) has given a graphical method to obtain single sampling plans for given values of ($p_1$, 1-$\alpha$) and ($p_2$, $\beta$) with the help of the poisson cumulative probability chart. Cameron (1952) has also given a table, which is an extension of the table given by Peach and Littauer (1946). Cameron's table is based on poisson distribution and can be used to design single sampling for all the popular values of producer's and consumer's risks.

Further tabularized $p_2/p_1$ , values for ($\alpha$ , $\beta$ ) = ( 0.05, 0.10), (0.05, 0.05). (0.05, 0.01). (0.01, 0.05), and ((0.01, 0.10) for c values ranging from 0 to 49. Using Cameron (1952) table, one can select a single sampling plan for given $p_1$ and $p_2$, $\alpha$ and $\beta$. Horsnell (1954) has also presented a table similar to that of Cameron, giving $p_2/p_1$ and $np_1$ values for $\alpha = 0.05$, 0.01 and $\beta$ = 0.10, 0.05, and 0.01 but restricting c from 1 to 20. Horsnell (1954) has further illustrated the approximation involved in replacing binomial probabilities by corresponding poisson probabilities by comparison of p values for $P_a$ (P) = 0.99, 0.95, 0.50, 0.10 and 0.01 for different single Sampling plans. Kirfkpatrick (1965) has given two tables for the selection of single sampling plans corresponding to different values of $p_1$ and $p_2$. The first table gives single sampling plans when OC curves pass very close to the specified. $p_1$ and not so close to the specified $p_2$ and the second table gives single sampling plan when OC curves pass very close to the specified $p_1$. The plans indexed are based on Grubbs (1948) tabulation of $p_1$ and $p_2$ for n = 1 (1) 50 and c = 0 (1) 9.

Guenther (1969) has developed a systematic research procedure for finding the single sampling plans for given $p_1$, $p_2$, $\alpha$ and $\beta$ based on the binomial, hyper geometric and poisson models. Hailey (1980) has presented a computer

program to obtain minimum sample size single sampling plans based on Guenther ( 1969) procedure for given $p_1$ ,$p_2$, $\alpha$ and $\beta$.

Stephens (1978) has given a procedure and tables for finding the samples size and acceptance number of a single sampling plan for given two points on the OC curve, viz,. ($p_1$, 1-$\alpha$) and ($p_2$, $\beta$) using normal approximation to binomial distribution. By using this procedure any point ($p_1$, 1-$\alpha$) and ($p_2$, $\beta$) may be specified and the applicable sample size and acceptance number can be found quite straight forward based on the formula for n. schilling and Johnson (1980) presented a set of tables for the construction and evolution of matched sets of single, double and multiple sampling plans. They may be used to derive two point individual plans to specified values of fraction defective and probability of acceptance.

Golub (1953) has given a method and tables for finding the acceptance number 'c' of a single sampling plan involving minimum sum of producer's and consumer's risks for given $p_1$ and $p_2$. when the sample size n is fixed. Soundararajan (1981) has extended the Golub's approach to single sampling plans when the conditions for application of the poisson model. Vijayathilakan (1982) has given procedure and tables for designing single sampling plans when the sample size is fixed and the sum of the weighted risks is minimized. Nirmala and Suresh (2017) presented a Continuous sampling plan indexed through maximum allowable average out going quality.

**Properties of OC Curve**

1) For a QSS (n; $c_N$, $c_T$) the steepness of the operating characteristic (OC) curve and hence its discriminating power is depending upon the difference between $c_N$ and $c_T$. For a fixed n, and fixed $c_N$, as $c_T$ decreases, the resulting composite OC curve gets steeper.
2) For a QSS(n,kn;$c_0$) for k>0, the slope of the composite OC curve increases as k increases. The conditions for application under which the Quick Switching System can be applied and the operating procedure are as follows:

**Conditions for Application**

1. The production is steady so that results on current and previous lots are broadly indicative of a ongoing process and submitted lots are excepted to be essentially of the same quality.
2. Lots are submitted considerably in the order of production.
3. Inspection is by characteristics with quality defined as fraction nonconforming.

**Operating Procedure of QSS (n, $c_N$, $c_T$)**

Step 1: From a lot, take a random sample of size 'n' at the normal level. Count the number of defectives 'd'.

i) If d $\leq c_N$, accept the lot and repeat step 1
ii) If d > $c_N$, reject the lot and go to step 2.

Step 2: From the next lot, take a random sample of size n at the tightened level. Count the number of defectives 'D".

i. If D $\leq c_T$, accept the lot and use step 1
ii. If D > $c_T$, reject the lot and repeat step 2

Romboski (1969) has introduced another sampling inspection system QSS-1 (n, kn; $c_0$) which is a QSS-1 with single sampling plan as a reference plan (n, $c_0$) and (kn, $c_0$), k>1 are respectively the normal and tightened single sampling plans. The conditions for application of this system are the same as that of QSS-1 (n: $c_N$, $c_T$ ).

**Operating procedure for QSS (n, kn, $c_0$)**

1. For a lot, take a random sample of size 'n' at the normal level. Count the number of defectives '

- If d$\leq c_0$, accept the lot and repeat step 1.
- If d > $c_0$, reject the lot and go to step 2.

2. From the next lot, take a random sample of size 'kn' at the tightened level. Count the number of defectives 'D'.

- If D≤ $c_0$, accept the lot and use step1
- If D> $c_0$, reject the lot and repeat step 2.

The OC function of the system is given in equation (1.2.1) with
$P_N$ – proportion of lots expected to be accepted when using ( n, $c_o$) plan
$P_T$ - proportion of lots expected to be accepted when using (kn, $c_o$) plan

Romboski (1969) has derived the OC function for QSS-1 (n, kn, $c_0$) as,

$$Pa(P) = \frac{P(d \le C_T; n)}{1 - P(d \le C_N; n) + P(d \le C_T; n)} \qquad (3)$$

**The Condition for application of Quick Switching System**

1. The production is steady so that results on current and preceding lots are broadly indicative of a ongoing process and submitted lots are expected to be fundamentally of the same quality.
2. Lots are submitted significantly in his order of production.
3. Inspection by qualities is considered with quality defined as fraction nonconforming 'p'.

**Operating Procedure for the plan**

1. Under the normal examination, inspect using the SSP plan with parameters n, $u_1$ and $u_2$. If a lot is accepted, continue with normal inspection. If a lot is rejected, go to step2.
2. Inspect under tightened examination using the SSP plan with sample size 'n' and acceptance number $v_1$ and $v_2$. If a lot is accepted, use step1 for the next lot, otherwise continue step2.

**Measures of Performance**
**Operating Characteristics function**

Based on Romboski (1965) and **Sankar and Mahopatra (1991)**, the expression for OC function of QSS-SSP is given by

$$Pa(p) = \frac{P(d \le c_T ; n)}{P(d \le c_N; n)} \qquad (4)$$

$$P(d \le c_N; n) = \frac{P_a(1-P_c)^i + P_c P_a^{\,i}}{(1-P_c)} \qquad (5)$$

Where $P_a$ = P [d≤ $u_1$] and $P_c$ = P [$u_1$<d<$u_2$]

$$P(d \le c_T; n) = \frac{P_a(1-P_c)^i + P_c P_a^{\,i}}{(1-P_c)} \qquad (6)$$

Where $P_a = P\,[d \le v_1]$ and $P_c = P\,[v_1 < d < v_2]$

**Palya Distribution**

$$PY_{N,m,r,c}(x) = \frac{\binom{r}{c}+x-1\ C\ x)\binom{N-r}{c}+n-x-1\ C\ n-x}{\frac{N}{c}+n-1Cn}\ , x = 0,1,2, \dots \dots, n. \quad (7)$$

Where N,n,r and c are natural numbers.
Using equation (4), the properties of the type B OC curves of QSS-SSP are analyzed and are given below:

Fig 1. Gives the OC curves of SSP (100; 2,4), QSS-SSP (100; 2, 4 ; 1, 2) and SSP (100; 1,2). From the figure it is observed that the system utilizes the normal SSP plan when the quality is good and the tightened SSP plan when the quality is poor. OC curves of QSS-SSP where the sample size and the acceptance numbers of the normal SSP plan are same whereas the acceptance numbers of the tightened SSP plan are allowed to decrease. As $v_1$ and $v_2$ become smaller, the discriminating power of the OC curve increases.

**Plotting the OC-curve of a given systems:**
Table 1 can be used to obtain nine values of 'p' and $P_a(P)$ to plot the OC-curve of a given QSS-1 (n, $k_n$;$c_o$). For example, for given QSS-1 (100,150;1,5) one gets k=150/100=1.5. Dividing the entries in the row corresponding to $u_1$=1, $u_2$=5 and k=1.5 of table 1 by normal Sample single 100, one gets the values of p and corresponding Pa(P) values are given in the column heading. The values of 'p' and $P_a(P)$ of QSS-1 (100,150;1,5).

**Designing the systems given Single Sampling Plan (SSP) and a point on the OC-curve**
Table 1 can be used to design QSS-SSP when the sample is fixed at 'n' and a point on the OC-curve (p, $P_a(P)$) is specified. To design a system, calculate 'np' and under the column $P_a(p)$ 0.99 in Table (1) find the value '0.473'. The $c_1$, $c_2$, $d_1$,and $d_2$ values corresponding to the selected tabular value together with the given n, determine the sampling system to be used. For example, let n =150, $p_1$=0.02 and Pa(p) =0.95 is 0.523. scan the column headed by Pa(p) =0.95, to find the value which is nearer to the desired value of 0.5, the value is 0.523 which corresponds to the parameters $c_1$ =0, $c_2$=1 $d_1$=0 and $d_2$=1 for the desired QSS-SSP.

## CONCLUSION

In acceptance sampling the producer and consumer plays a leading role and hence one allows a certain level of risk for producer and consumer. It is comprehensible to design any sampling plan with the accompanying quality levels, concern to producer and consumer in practice. Hence selection measures are considered in this paper with inflection point on the OC curve. Tables provided in this paper are tailor-made which are handy and ready-made, which are also well considered for assessment purposes.

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**Table 1. Plotting the OC-curve of a given systems**

| P | 0.0115 | 0.0152 | 0.01731 | 0.0204 | 0.05413 | 0.02416 | 0.03712 | 0.04613 | 0.06521 |
|---|---|---|---|---|---|---|---|---|---|
| Pa(P) | 0.99 | 0.95 | 0.90 | 0.75 | 0.50 | 0.25 | 0.10 | 0.05 | 0.01 |

**Table 2 $P_a(p)$ values for QSS-1 with SSP plan as a reference plan for (i, $c_N$, $c_T$)**

| $C_N$ | | $C_T$ | | i | 0.99 | 0.950 | 0.9 | 0.75 | 0.5 | 0.25 | 0.1 | 0.05 | 0.01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_1$ | $C_2$ | $D_1$ | $D_2$ | | | | | | | | | | |
| 0 | 1 | 0 | 1 | 2 | 0.473 | 0.523 | 0.673 | 0.873 | 1.123 | 1.423 | 1.773 | 2.173 | 2.623 |
| | | | | 4 | 0.317 | 0.367 | 0.517 | 0.717 | 0.967 | 1.267 | 1.617 | 2.017 | 2.467 |
| | | | | 6 | 0.251 | 0.301 | 0.451 | 0.651 | 0.901 | 1.201 | 1.551 | 1.951 | 2.401 |
| | | | | 8 | 0.212 | 0.262 | 0.412 | 0.612 | 0.862 | 1.162 | 1.512 | 1.912 | 2.362 |
| | | | | 10 | 0.181 | 0.231 | 0.381 | 0.581 | 0.831 | 1.131 | 1.481 | 1.881 | 2.331 |
| 0 | 1 | 0 | 1 | 2 | 0.48 | 0.530 | 0.68 | 0.88 | 1.13 | 1.43 | 1.78 | 2.18 | 2.63 |
| | | | | 4 | 0.321 | 0.371 | 0.521 | 0.721 | 0.971 | 1.271 | 1.621 | 2.021 | 2.471 |
| | | | | 6 | 0.252 | 0.302 | 0.452 | 0.652 | 0.902 | 1.202 | 1.552 | 1.952 | 2.402 |
| | | | | 8 | 0.21 | 0.260 | 0.41 | 0.61 | 0.86 | 1.16 | 1.51 | 1.91 | 2.36 |
| | | | | 10 | 0.185 | 0.235 | 0.385 | 0.585 | 0.835 | 1.135 | 1.485 | 1.885 | 2.335 |
| 1 | 1 | 0 | 1 | 2 | 1.267 | 2.317 | 3.467 | 4.667 | 5.917 | 7.217 | 8.567 | 9.967 | 11.41 |
| | | | | 4 | 1.015 | 1.065 | 1.215 | 1.415 | 1.665 | 1.965 | 2.315 | 2.715 | 3.165 |
| | | | | 6 | 0.846 | 0.896 | 1.046 | 1.246 | 1.496 | 1.796 | 2.146 | 2.546 | 2.996 |
| | | | | 8 | 0.788 | 0.838 | 0.988 | 1.188 | 1.438 | 1.738 | 2.088 | 2.488 | 2.938 |
| | | | | 10 | 0.724 | 0.774 | 0.924 | 1.124 | 1.374 | 1.674 | 2.024 | 2.424 | 2.874 |
| 1 | 1 | 0 | 0 | 2 | 1.327 | 2.377 | 3.527 | 4.727 | 5.977 | 7.277 | 8.627 | 10.02 | 11.47 |
| | | | | 4 | 1.032 | 1.082 | 1.232 | 1.432 | 1.682 | 1.982 | 2.332 | 2.732 | 3.182 |
| | | | | 6 | 0.858 | 0.908 | 1.058 | 1.258 | 1.508 | 1.808 | 2.158 | 2.558 | 3.008 |
| | | | | 8 | 0.794 | 0.844 | 0.994 | 1.194 | 1.444 | 1.744 | 2.094 | 2.494 | 2.944 |
| | | | | 10 | 0.732 | 0.782 | 0.932 | 1.132 | 1.382 | 1.682 | 2.032 | 2.432 | 2.882 |
| 0 | 1 | 1 | 0 | 2 | 2.173 | 2.223 | 2.373 | 2.573 | 2.823 | 3.123 | 3.473 | 3.873 | 4.323 |
| | | | | 4 | 1.788 | 1.838 | 1.988 | 2.188 | 2.438 | 2.738 | 3.088 | 3.488 | 3.938 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 6 | 1.632 | 1.682 | 1.832 | 2.032 | 2.282 | 2.582 | 2.932 | 3.332 | 3.782 |
| | | | | 8 | 1.48 | 1.530 | 1.68 | 1.88 | 2.13 | 2.43 | 2.78 | 3.18 | 3.63 |
| | | | | 10 | 1.388 | 1.438 | 1.588 | 1.788 | 2.038 | 2.338 | 2.688 | 3.088 | 3.538 |
| 0 | 1 | 1 | 0 | 2 | 2.174 | 2.224 | 2.374 | 2.574 | 2.824 | 3.124 | 3.474 | 3.874 | 4.324 |
| | | | | 4 | 1.814 | 1.864 | 2.014 | 2.214 | 2.464 | 2.764 | 3.114 | 3.514 | 3.964 |
| | | | | 6 | 1.628 | 1.678 | 1.828 | 2.028 | 2.278 | 2.578 | 2.928 | 3.328 | 3.778 |
| | | | | 8 | 1.482 | 1.532 | 1.682 | 1.882 | 2.132 | 2.432 | 2.782 | 3.182 | 3.632 |
| | | | | 10 | 1.399 | 1.449 | 1.599 | 1.799 | 2.049 | 2.349 | 2.699 | 3.099 | 3.549 |
| 0 | 1 | 1 | 0 | 2 | 2.219 | 2.269 | 2.419 | 2.619 | 2.869 | 3.169 | 3.519 | 3.919 | 4.369 |
| | | | | 4 | 1.825 | 1.875 | 2.025 | 2.225 | 2.475 | 2.775 | 3.125 | 3.525 | 3.975 |
| | | | | 6 | 1.625 | 1.675 | 1.825 | 2.025 | 2.275 | 2.575 | 2.925 | 3.325 | 3.775 |
| | | | | 8 | 1.493 | 1.543 | 1.693 | 1.893 | 2.143 | 2.443 | 2.793 | 3.193 | 3.643 |
| | | | | 10 | 1.402 | 1.452 | 1.602 | 1.802 | 2.052 | 2.352 | 2.702 | 3.102 | 3.552 |
| 1 | 1 | 1 | 0 | 2 | 2.272 | 3.322 | 4.472 | 5.672 | 6.922 | 8.222 | 9.572 | 10.97 | 12.42 |
| | | | | 4 | 1.829 | 1.879 | 2.029 | 2.229 | 2.479 | 2.779 | 3.129 | 3.529 | 3.979 |
| | | | | 6 | 1.625 | 1.675 | 1.825 | 2.025 | 2.275 | 2.575 | 2.925 | 3.325 | 3.775 |
| | | | | 8 | 1.493 | 1.543 | 1.693 | 1.893 | 2.143 | 2.443 | 2.793 | 3.193 | 3.643 |
| | | | | 10 | 1.401 | 1.451 | 1.601 | 1.801 | 2.051 | 2.351 | 2.701 | 3.101 | 3.551 |
| 1 | 1 | 1 | 0 | 2 | 2.987 | 4.037 | 5.187 | 6.387 | 7.637 | 8.937 | 10.28 | 11.68 | 13.13 |
| | | | | 4 | 2.595 | 2.645 | 2.795 | 2.995 | 3.245 | 3.545 | 3.895 | 4.295 | 4.745 |
| | | | | 6 | 2.37 | 2.420 | 2.57 | 2.77 | 3.02 | 3.32 | 3.67 | 4.07 | 4.52 |
| | | | | 8 | 2.227 | 2.277 | 2.427 | 2.627 | 2.877 | 3.177 | 3.527 | 3.927 | 4.377 |
| | | | | 10 | 2.102 | 2.152 | 2.302 | 2.502 | 2.752 | 3.052 | 3.402 | 3.802 | 4.252 |
| $C_N$ | | $C_T$ | | i | 0.99 | 0.95 | 0.9 | 0.75 | 0.5 | 0.25 | 0.1 | 0.05 | 0.01 |
| $C_1$ | $C_2$ | $C_1$ | $C_2$ | | | | | | | | | | |
| 0 | 1 | 0 | 1 | 2 | 3.187 | 3.237 | 3.337 | 3.487 | 3.687 | 3.937 | 4.237 | 4.587 | 4.987 |
| | | | | 4 | 2.692 | 2.742 | 2.842 | 2.992 | 3.192 | 3.442 | 3.742 | 4.092 | 4.492 |
| | | | | 6 | 2.543 | 2.593 | 2.693 | 2.843 | 3.043 | 3.293 | 3.593 | 3.943 | 4.343 |
| | | | | 8 | 2.255 | 2.305 | 2.405 | 2.555 | 2.755 | 3.005 | 3.305 | 3.655 | 4.055 |
| | | | | 10 | 2.137 | 2.187 | 2.287 | 2.437 | 2.637 | 2.887 | 3.187 | 3.537 | 3.937 |
| 0 | 1 | 0 | 1 | 2 | 3.94 | 3.99 | 4.091 | 4.241 | 4.441 | 4.690 | 4.990 | 5.341 | 5.740 |
| | | | | 4 | 3.45 | 3.501 | 3.601 | 3.750 | 3.95 | 4.201 | 4.501 | 4.850 | 5.251 |
| | | | | 6 | 3.203 | 3.253 | 3.353 | 3.503 | 3.703 | 3.953 | 4.253 | 4.603 | 5.003 |
| | | | | 8 | 3.024 | 3.074 | 3.174 | 3.324 | 3.524 | 3.774 | 4.074 | 4.424 | 4.824 |
| | | | | 10 | 2.894 | 2.944 | 3.044 | 3.194 | 3.394 | 3.644 | 3.944 | 4.294 | 4.694 |
| 1 | 1 | 0 | 1 | 2 | 4.025 | 5.075 | 6.175 | 7.325 | 8.525 | 9.775 | 11.075 | 12.425 | 13.82 |
| | | | | 4 | 3.572 | 3.622 | 3.722 | 3.872 | 4.072 | 4.322 | 4.622 | 4.972 | 5.372 |
| | | | | 6 | 3.218 | 3.268 | 3.368 | 3.518 | 3.718 | 3.968 | 4.268 | 4.618 | 5.018 |
| | | | | 8 | 3.036 | 3.086 | 3.186 | 3.336 | 3.536 | 3.786 | 4.086 | 4.436 | 4.836 |
| | | | | 10 | 2.894 | 2.944 | 3.044 | 3.194 | 3.394 | 3.644 | 3.944 | 4.294 | 4.694 |
| 1 | 1 | 0 | 0 | 2 | 4.248 | 5.298 | 6.398 | 7.548 | 8.748 | 9.998 | 11.29 | 12.64 | 14.04 |
| | | | | 4 | 3.51 | 3.560 | 3.660 | 3.810 | 4.010 | 4.260 | 4.560 | 4.910 | 5.310 |
| | | | | 6 | 3.264 | 3.314 | 3.414 | 3.564 | 3.764 | 4.014 | 4.314 | 4.664 | 5.064 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 8 | 3.037 | 3.087 | 3.187 | 3.337 | 3.537 | 3.787 | 4.087 | 4.437 | 4.837 |
| | | | | 10 | 2.896 | 2.946 | 3.046 | 3.196 | 3.396 | 3.646 | 3.946 | 4.296 | 4.696 |
| 0 | 1 | 1 | 0 | 2 | 4.783 | 4.833 | 4.933 | 5.083 | 5.283 | 5.533 | 5.833 | 6.183 | 6.583 |
| | | | | 4 | 4.281 | 4.331 | 4.431 | 4.581 | 4.781 | 5.031 | 5.331 | 5.681 | 6.081 |
| | | | | 6 | 4.214 | 4.264 | 4.364 | 4.514 | 4.714 | 4.964 | 5.264 | 5.614 | 6.014 |
| | | | | 8 | 3.810 | 3.861 | 3.961 | 4.110 | 4.311 | 4.561 | 4.861 | 5.211 | 5.611 |
| | | | | 10 | 3.657 | 3.707 | 3.807 | 3.957 | 4.157 | 4.407 | 4.707 | 5.057 | 5.457 |
| 0 | 1 | 1 | 0 | 2 | 4.874 | 4.924 | 5.024 | 5.174 | 5.374 | 5.624 | 5.924 | 6.274 | 6.674 |
| | | | | 4 | 4.331 | 4.381 | 4.481 | 4.631 | 4.831 | 5.081 | 5.381 | 5.731 | 6.131 |
| | | | | 6 | 4.031 | 4.081 | 4.181 | 4.331 | 4.531 | 4.781 | 5.081 | 5.431 | 5.831 |
| | | | | 8 | 3.822 | 3.872 | 3.972 | 4.122 | 4.322 | 4.572 | 4.872 | 5.222 | 5.622 |
| | | | | 10 | 3.667 | 3.717 | 3.817 | 3.967 | 4.167 | 4.417 | 4.717 | 5.067 | 5.467 |
| 0 | 1 | 1 | 0 | 2 | 4.555 | 4.605 | 4.705 | 4.855 | 5.055 | 5.305 | 5.605 | 5.955 | 6.355 |
| | | | | 4 | 4.345 | 4.395 | 4.495 | 4.645 | 4.845 | 5.095 | 5.395 | 5.745 | 6.145 |
| | | | | 6 | 4.008 | 4.058 | 4.158 | 4.308 | 4.508 | 4.758 | 5.058 | 5.408 | 5.808 |
| | | | | 8 | 3.835 | 3.885 | 3.985 | 4.135 | 4.335 | 4.585 | 4.885 | 5.235 | 5.635 |
| | | | | 10 | 3.672 | 3.722 | 3.822 | 3.972 | 4.172 | 4.422 | 4.722 | 5.072 | 5.472 |
| 1 | 1 | 1 | 0 | 2 | 4.975 | 6.025 | 7.12 | 8.275 | 9.475 | 10.72 | 12.02 | 13.37 | 14.77 |
| | | | | 4 | 4.37 | 4.42 | 4.52 | 4.67 | 4.87 | 5.12 | 5.42 | 5.77 | 6.17 |
| | | | | 6 | 4.051 | 4.101 | 4.201 | 4.351 | 4.551 | 4.801 | 5.101 | 5.451 | 5.851 |
| | | | | 8 | 3.843 | 3.893 | 3.993 | 4.143 | 4.343 | 4.593 | 4.893 | 5.243 | 5.643 |
| | | | | 10 | 3.686 | 3.736 | 3.836 | 3.986 | 4.186 | 4.436 | 4.736 | 5.086 | 5.486 |

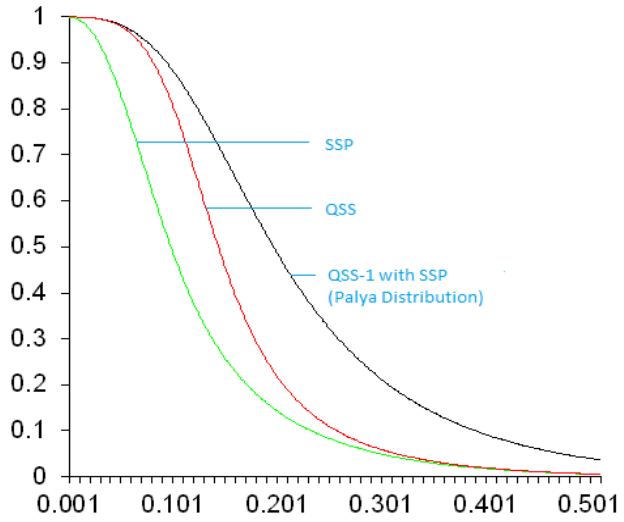


**Fig.1: Quick Switching System (QSS-1) with Single Sampling (SSP) Plan with Palya Distribution**

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**RESEARCH ARTICLE**

# Heavy Metal Contamination of Soil: A Tractable Study in Gold Tailings Area of Kolar Gold Fields, India- using ICP-0ES and AES

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## ABSTRACT

The study has a dual objective, firstly to determine the concentration of arsenic (As) in the soil and ground water within the vicinity of a historical gold mine region located in the Kolar Gold fields (KGF), Karnataka. The study employed Thermo Scientific's Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and ICP-AES using Iteva software to analyze the distribution of arsenic in soil and ground water. Four soil and ground water samples were collected from the selected study sites (A, B, C and D) proximity to residential area, were examined from January - December 2023. The analysis of soil samples collected from the four mine landfills revealed elevated levels of heavy metal. The highest level of arsenic that can be present in the soil is 12 mg/kg (WHO 2008). The highest average arsenic content in the soil samples during January-December 2023 analyzed from Champion was 120.83 mg/kg, site A (36.08 mg/kg), D (32.17 mg/kg), and B (28.00 mg/kg) respectively. The maximum permissible limit of arsenic in groundwater is 0.05 mg/L (USEPA 2009). The ground water analysis revealed samples highly contaminated with arsenic (0.863 mg/L) in site C (Champion). The average lead content is found to be 0.559 mg/L, at sites B, A and D is 0.323 mg/L, B, 0.277 mg/L 0.161 mg/L respectively. The pH and electrical conductivity of all the water samples were within the permissible limits, but hardness of the ground water was two times higher than the permissible limit in the sample site D (Balghat).

**Keywords :** Soil pollution, Ground water pollution, Lead, ICP-OES, ICP-AES.

## INTRODUCTION

There have been momentous global environmental emissions of hazardous chemicals as a result of growing urbanization and industry [1, 2]. While some of these poisons are naturally occurring, human causes, particularly mining operations, have significantly accelerated their growth. Despite having a sizable positive social and economic impact on a country, it is difficult to ignore the mining's long-term detrimental consequences on the public health and the [3]. Over the past few decades, urbanization has rapidly increased due to the tremendous expansion in the human population. Due to the fact that untreated wastewater discharge pollutes water bodies and promotes diseases related to water, the correct management of the enormous amount of urban wastewater is an international challenge the [4]. The town of Kolar Gold Fields, often known as K.G.F., is located in the Bangarpet taluk of the Kolar district in the Indian state of Karnataka. In order to take over the mining operations of the mines situated at latitude 12° 53'12" N and longitude 78°15'03" E, at the southernmost point of a short schist strip of the township, KGF, whose residents are primarily the families of gold mine workers, the Government of India established the public company (Bharat Gold Mines Limited), or BGML. For more than a century, underground gold mining has been practiced at KGF. 65 kilometers of tunnel construction have been used to mine gold to a depth of 3 kilometers below the surface, and 40 million tons of mill tailings have accumulated the [5].

Metallurgical extraction must first break the crystallographic connections in order to extract the required element or compound from the ore source the [6].This process creates a considerable amount of waste in the case of gold mining, when practically all of the ore obtained is discarded the [7]. After the valuable ore has been extracted during the mining process, the leftover finely powdered rock and water are known as tailings. There are significant amounts of tailings that have been discarded in the open in many nations where environmental restrictions are not effectively implemented. Tailings particles have a similar chemical and physical makeup to similar river sand and mud the [8].
Tailings, a by-product of gold extraction that is heavily polluted with heavy metals, are the main product (HM) the [9, 10].These metals seep out into the environment uncontrollably when they come into touch with water or are dispersed by the wind the [11]. The quality of both surface and groundwater is significantly impacted by surface impacts like tailings and rock dumps. The process of using cyanide to extract gold from ore, also contributes to global warming, hydrogen cyanide emissions, and the creation of vast number of tailings, which might be a source of heavy metals (HMs) the [12].

The aforementioned factors are only a few of the many sources of tailing characteristics. Sediments from mines frequently resemble a particular type of river sand or silt in terms of their physical and chemical characteristics. The geochemistry, makeup, and mineralogy of the ore, as well as the procedures used to extract different commercial goods, all influence the specific features of tailings. Chemically, gold tailings are highly salinized, include just 6% pyrite, and contain very little organic stuff. According to sources, the pH of Iran's tailings was 7.35, that of South Africa's was 3.25-6.28, and that of India was 3.48-8.12 the [13]. Heavy metal soil pollution is caused in the regions with gold mining activities primarily due to the extraction of ores from soil and rocks and due to unscientific methods of dumping the leftover tailings around residential places. Heavy metals may endanger humans, animals, plants, and the ecosystem through various channels the [14, 15, 16].These include direct consumption, plant absorption, food chains, ingesting polluted water, and changes to the pH, opacity, color, all of which affect soil quality the [17].

Even though heavy metals are only found in trace concentrations in water sources, they are exceedingly dangerous and pose serious health hazards to both people and other species the [18, 19]. When humans consume toxic metals in excess, it can cause serious stomach pain, extreme vomiting, and deteriorating liver abnormalities. Arsenic poisoning from drinking water has been linked to horrible long-term effects, such as learning issues, nervous system damage, and children's growth being stunted the [20]. Gradually the heavy metals present in soil tends to get washed off from tailings present in high altitude during monsoon season and seeps into groundwater, lakes, and pond water. The HMs which are present in pond water tend to invariably affect the metabolism and growth of the fish. Fishes

containing impermissible levels of heavy metals when consumed by animals in higher tropic levels get affected resulting in various complications. The study attempted to demonstrate that heavy metal concentrations in Channa punctatus from sewage-fed aquaculture ponds in India constitute a health threat to locals. The majority of the fish samples examined contained dangerously high amounts of heavy metals, putting the local population at risk if they consumed too much fish [21]. Over the course of a century, various companies mined gold. Low gold production and unprofitability forced its closure in 2001 the [22]. Heavy metal pollution comes from mining. Mining contaminates soil, generating environmental hazards. Samples contained heavy metals in soil and water the [23]. Heavy metals can cause cancer, organ damage, stunted growth, and death the [24].Heavy metal removal from soils requires remediation. He concluded that rainwater penetration contaminates groundwater, which contaminates the soil. Heavy metal harms plants, animals, and humans. Thus, it is vital to assess soil contamination and offer remedial actions to improve soil quality and reduce contamination.

In the majority of the world's accessible freshwater reservoirs, groundwater makes up 99 percent of the planet's liquid freshwater supply. Since it can be pumped, groundwater often becomes the main source of water in areas where there are no other permanent water supplies. Currently, groundwater is the source of over 50% of all drinking water, 40% of all agricultural water, and 35% of all industrial the [25]. The main contributors to heavy metal contamination have been Arsenic (As), chromium (Cr), mercury (Hg), and cadmium (Cd). The presence of these metals has caused a number of issues for plant life in the beautiful surroundings. In growing plants, cadmium causes leaf chlorosis, or inadequate chlorophyll. Plants that contain excessive levels of arsenic and mercury have experienced a decrease in photosynthesis and root development. Chromium is not biodegradable and significantly reduces the dry weight of seedlings. They enter the food chain and accumulate at various trophic levels, which have an adverse influence on the growth of plants the [26].The immune system is altered by cadmium, and proliferative prostatic lesions and lung adenocarcinoma are more common. Overexposure to blood arsenic can lower a child's intellectual capacity, damage their endocrine, skeletal, and immune systems, and induce hypertension. In adults, it also impairs cardiac and renal function. Chromium has been connected to respiratory system tumors and cancer the [27]. Groundwater contamination from rainwater infiltration eventually contaminates soil the [22]. Heavy metal harms plants, animals, and humans. Thus, it is vital to assess pollution and offer remedial strategies to improve groundwater quality and reduce contamination. Six water samples from bore wells (BWs) were chosen at random from the mining and residential regions of KGF. To evaluate the presence of heavy metals in the KGF, samples of bore well (BW) water were taken all throughout the research area. Six samples of well water were examined in the lab. Heavy elements like copper (Cu), nickel (Ni), and arsenic (As) were discovered in the water during the preliminary research.

## COLLECTION OF SOIL SAMPLES

### General methodology

During this research work, the general methodology was devised based on spatiotemporal factors like place and time. So, the four places selected based on the minimal distance from the abandoned gold mining sites were Oorgaum, Tenants, Champion, and Balghat and named A, B, C, and D which are located 50,100, 200 and 300 meters from the mining sites respectively.

The four sampling seasons were

Season 1-January, February, and March

Season 2-April, May, and June

Season 3- July, August, and September

Season 4-October, November, and December

Four sample locations were used for the sampling, which took place between January and December 2023 (gold ore tailings). Every month, one sample was taken from each location, resulting in four samples that were examined. The presence of certain heavy metals, such as Cd, Cr, and Pb was determined by sampling the area throughout the four distinct seasons. After removing contaminants from the surface, soil samples were collected from 10 to 20 cm below the surface and put in self-locking polythene bags. The sample preparation procedures for the spectrochemical

determination of the total recoverable elements were finished in accordance with U.S. Environmental Protection Agency requirements the [28].

**Sample preparation**

To calculate the total recoverable analytes in soil samples, the sample was well mixed, a piece of it was placed on the tared weighing dish, the sample was weighed, and the weight was recorded in order to ascertain the total recoverable analytes in solid samples. The dry material was mashed in a mortar and pestle and sieved using a 5-mesh propylene sieve to obtain homogeneity. All of the acids and chemicals utilised in this research were ultra-purity grade, and Rankem Compounds provided the chemicals for measurement. Thermo Scientific's ICP-OES (Inductively Coupled Plasma- Optical Emission Spectroscopy) was used in conjunction with Iteva software to evaluate the samples.

**Sample digestion and analysis**

As reagents, Rankem chemicals' pure analytical grade acids with a 32 percent HCl content and a 70 percent $HNO_3$ content (both purchased from Sigma-Aldrich India) were employed. Concentrated $HNO_3$ and HCl acids were combined in a 1:3 ratio to create aqua-regia. In a 250 mL conical glass flask, one gramme of each reference material or dry powdered soil was combined with 28 mL of aqua-regia. The reactants were gently mixed in the flask before it was heated on a hot plate for five hours, reaching a temperature of 120°C. The dissolved samples were filtered into 100 mL HDPE bottles after cooling down using filter paper that had been wetted with 3 percent $HNO_3$ acid for ICP-OES analysis. All glassware used to prepare samples was cleaned before use by immersing it in an acid solution containing 10 percent v/v $HNO_3$ for 24 hours. This was done before giving the glassware a final rinse with deionized distilled water. Thermo Fischer, ICP-OES, and iCAP 6300 were used to perform chemical analyses at the ARML in Bangalore, India, and ITEVA software was used to interpret the results.

## RESULTS AND DISCUSSION

Arsenic **concentration in soil**

Arsenic, as a heavy metal, is known for its stability. In both animals and humans, it acts as a neurotoxin. The soil may contain trace quantities of arsenic. One of the most pervasive and deadly forms of pollution in the world is arsenic (AS) pollution. The highest level of arsenic that can be present in the soil is 98 mg/kg(Table 1). In site (C) Champion, the amount of Arsenic (As) is 140 mg/kg, which is almost 1.2 times the upper limit set by the [29] as permissible limits. The highest average Arsenic content in the soil samples during January-December 2023 analyzed from Champion was 120.83 mg/kg, followed by 36.08 mg/kg at site A, 32.17 mg/kg at site D, and 28.00 mg/kg at site B. Tenants' soil contamination in season 3 was (19 mg/kg) as the lowest concentration through the campaign. When assessed season-wise in all sample sites the average concentrations season-wise was, season 4 at 60.45 mg/kg, followed by season 3 at 56.3 mg/kg, season 1 had 51.9 mg/kg, and the lowest amount observed during season 2 at 48.3 mg/kg.

In the graph 1, sites A (Oorgaum), B (Tenants), and D (Balghat) all have Arsenic levels in their soil that are below the legal limit. Only site C (Champion) shows higher Arsenic values in the soil sample than the permissible limits. The Arsenic concentrations in the soil samples are slightly rising from season to season. Initial concentrations tend to be lowest during season 1, with subsequent concentrations gradually rising throughout the year. According to Table 2, there is a positive and significant correlation between Arsenic pollution in the soil during seasons 1 (January–March) and 2 ($r = 1.00$, $p < 0.05$). There is also evidence of a statistically significant association concerning Arsenic pollution in soil, with values of ($r = 0.993$, $p < 0.05$) and ($r = 0.999$, $p < 0.05$), respectively, for Seasons 3 and 4.

**Table 3.** displays the results of a statistical analysis comparing arsenic concentrations in the soil across different seasons. The results show that there is a statistically significant difference between the arsenic concentrations in the soil between seasons 1 (January–March) and 4 (October–December) with a p-value of 0.003. We cannot statistically

report a difference in arsenic levels between seasons 1 (January–March) and 2 (April–June) and seasons 1 (January–March) and 3 (July-September) since the p-value is greater than 0.05.

**Effect of gold ore tailings on the groundwater during January-December 2023**

**Assessment of pH in bore well water**

An indicator of many geochemical equilibrium or solubility calculations, pH is widely recognized as a critical ecological component. Most aquatic creatures have adapted to a specific pH level and cannot survive if that level suddenly changes, making pH a crucial element in any body of water.

The limit pH value for drinking water is specified as 6.5 to 8.5 (table-4). Sampling site Balghat has the lowest pH value i.e., 6.6 and Champion's pH value is the highest among all the sample sites (7.4) followed by Tenants and Oorgaum with the pH values 7.1 and 6.8 respectively.

**Assessment of Hardness in groundwater**

The levels of calcium and magnesium, and to a lesser extent iron, are what determine the hardness of water. Water hardness is expressed as the amount of calcium carbonate ($CaCO_3$) in milligrams per liter (mg/L) by adding the calcium and magnesium concentrations together. The weathering of limestone, sedimentary rock, and calcium-bearing minerals gives most groundwater its hardness. Chemical and mining wastewater, as well as the overuse of lime as a soil amendment in agricultural regions, can all contribute locally to groundwater hardness. The optimum range of hardness in drinking water is 250 ppm to 660 ppm(table-5). All the other sampling site's borewell water's hardness is much higher than the permissible limits. The highest water hardness is seen in the Oorgaum borewell water sample, followed by sites D, C, and B (Graph-2).

**Arsenic concentration in groundwater**

The environment contains naturally occurring arsenic, a hazardous element. Often used in household items, although its content in the environment may have been raised by human activity; it is released into the atmosphere through vehicle exhaust. Pipe corrosion is a major source of this substance in drinking water. Children under the age of six are particularly vulnerable to the adverse health effects of arsenic exposure, even from relatively modest exposure levels. hemoglobin production is disrupted; blood pressure is raised; kidneys are damaged. In table-6, the highest level of arsenic that can be present in groundwater is 0.05 mg/L. Sampling site C Champion is highly contaminated with arsenic. In Champion, the content is 0.863 mg/L, which is much higher than the upper limit set by the WHO (2004) for safe consumption. The average arsenic content in the groundwater samples taken from Champion was 0.559 mg/L, followed by 0.323 mg/L at site B, 0.277 mg/L at site A, and 0.161 mg/L at site D. Balghat's groundwater contamination in season 3 and 4 (0.155 mg/L) was lowest.

Sites A (Oorgaum), C, and D (all shown on the graph 3) all have arsenic levels in their groundwater that are above the legal limit. Only site B shows constant arsenic values in the groundwater sample. The arsenic concentrations in the groundwater samples are slightly rising from season to season. Initial concentrations tend to be lowest during season 1, with subsequent concentrations gradually rising throughout the year. According to Table 8, there is a positive and significant correlation between arsenic pollution in groundwater during seasons 1 (January–March) and 2 ($r = 1.00$, $p < 0.05$). There is also evidence of a statistically significant association concerning arsenic pollution in groundwater, with values of ($r = 0.972$, $p < 0.05$) and ($r = 0.983$, $p < 0.05$), respectively, for Seasons 3 and 4.

In table 9, there was a statistically non-significant average difference between arsenic levels in the groundwater during season1 (Jan-Mar) & season 2 (Apr-Jun), season 1 (Jan-Mar) & season 3 (Jul-Sept) and season 1 (Jan-Mar) & season 4 (Oct-Dec) since the p-value >0.05.

## CONCLUSSION

The permissible pH value for drinking water is specified as 6.5 to 8.5. Sampling site Balghat has the lowest pH value i.e. 6.6 and Champion's pH value is the highest among all the sample sites (7.4) followed by Tenants and Oorgaum with the pH values 7.1 and 6.8 respectively. In a similar study in Singapore, the [30], the pH value of water was observed as 7.8±0.4. In a similar study in Kuwait, the pH value of water was observed in the range 7.33–7.45, justifying our results. The highest EC in borewell water is observed in Champion (2110 μS/cm), followed by Tenants, Oorgaum, and Balghat with the values 1900 μS/cm, 1850 μS/cm, and 1810 μS/cm respectively. In a similar study in Palestine the [31] demonstrated the EC value of water was observed in the range of 473–1406, justifying our obtained results. In contrast, a study in Wondo Genet, the [32], reported the EC value of water was observed as 192.14 μS/cm.
All the other sampling site's borewell water's hardness is much higher than the permissible limits. Hardness is almost 2 times higher than the control water hardness. The highest water hardness is seen in the Oorgaum borewell water sample, followed by sites D, C, and B. In a similar study in Europe, the [33]. reported the hardness value of water was observed as 60 ppm. In a similar study in Sri Lanka, the [34]. reported the hardness of groundwater as 385 ppm.

When assessed for arsenic in soil, sample site (C) Champion, contained 140 mg/kg of Pb, which is almost 1.2 times the upper limit set by the WHO (2008) as permissible limits. The highest average arsenic content in the soil samples during January-December 2023 analyzed from Champion was 120.83 mg/kg, followed by 36.08 mg/kg at site A, 32.17 mg/kg at site D, and 28.00 mg/kg at site B. Tenants' soil contamination in season 3 was (19 mg/kg) as the lowest concentration through the campaign. A similar study done in Karnataka the [35] found the arsenic content in soils from 30 mg kg−1 in Kiradalli Tanda village soil. Sampling site C Champion is highly contaminated with arsenic. In Champion, the content is 0.863 mg/L, which is much higher than the upper limit set by the [29].for safe consumption. The average arsenic content in the groundwater samples taken from Champion was 0.559 mg/L, followed by 0.323 mg/L at site B, 0.277 mg/L at site A, and 0.161 mg/L at site D. Balghat's groundwater contamination in season 3 and 4 (0.155 mg/L) was lowest. In a similar study in Taiwan, it was found that the arsenic values were found in the range 0.001-0.019 mg/L. the [36]. validated that in Iran, the arsenic concentration was found to be in the range of 1-4.23 mg/L.

**CONFLICTS OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1: Assessment of Arsenic in the soil (mg/kg) in various sampling sites during January-December 2023**

| SAMPLING SITE | MAXIMUM PERMISSBLE LIMIT | HIGHEST | LOWEST | AVERAGE |
|---|---|---|---|---|
| **OORGAUM (A)** | 98 | 44 | 28 | 36.08 |
| **TENANTS (B)** | | 34 | 19 | 28.00 |
| **CHAMPION (C)** | | 140 | 88 | 120.83 |
| **BALGHAT (D)** | | 44 | 21 | 32.17 |

**Table 2: Correlation of Arsenic concentration in Soil in various seasons**

| Seasons during 2023 | | Correlation | Sig. |
|---|---|---|---|
| 1 | Season 1 & 2 | 1.000 | .000 |
| 2 | Season 1 & 3 | .993 | .007 |
| 3 | Season 1 & 4 | .999 | .001 |

**Table 3: Comparison of Arsenic concentration in Soil in various seasons**

| Seasons | Paired Differences | | | | | t | df | P-value |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | Lower | Upper | | | |
| 1-2 | 3.58333 | 14.72306 | 7.36153 | -19.84434 | 27.01101 | .487 | 3 | .660 |
| 1-3 | -4.41667 | 6.08809 | 3.04404 | -14.10417 | 5.27084 | -1.451 | 3 | .243 |
| 1-4 | -8.58333 | 1.91243 | .95622 | -11.62644 | -5.54022 | -8.976 | 3 | .003 |

**Table 4: Average concentration of pH in groundwater in sampling sites 2023**

| Sites | pH |
|---|---|
| Max permissible limit | 6.5-8.5 |
| Oorgaum | 6.8 |
| Tenants | 7.1 |
| Champion | 7.4 |
| Balghat | 6.6 |

**Table 5: Average concentration of Hardness in groundwater in various sampling sites in 2023**

| Sampling Sites | Hardness in ppm |
|---|---|
| Max permissible limit | 250-660 |
| Oorgaum | 780 |
| Tenants | 690 |
| Champion | 710 |
| Balghat | 730 |

**Table 6: Concentration of Arsenic in groundwater in mg/L in sampling sites during January-December 2023**

| SAMPLING SITE | MAXIMUM PERMISSBLE LIMIT | HIGHEST | LOWEST | AVERAGE |
|---|---|---|---|---|
| **OORGAUM (A)** | 0.01 | 0.015 | 0.013 | 0.014 |
| **TENANTS (B)** | | 0.005 | 0.003 | 0.004 |
| **CHAMPION (C)** | | 0.349 | 0.157 | 0.228 |
| **BALGHAT (D)** | | 0.070 | 0.050 | 0.060 |

**Table 7: Concentration of arsenic in groundwater in mg/L in sampling sites during January-December 2023**

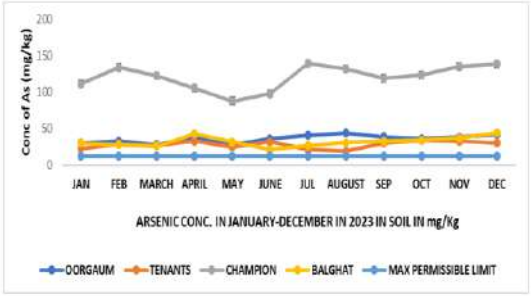
| SAMPLING SITE | MAXIMUM PERMISSBLE LIMIT | HIGHEST | LOWEST | AVERAGE |
|---|---|---|---|---|
| **OORGAUM (A)** | 0.05 | 0.283 | 0.271 | 0.277 |
| **TENANTS (B)** | 0.05 | 0.326 | 0.320 | 0.323 |
| **CHAMPION (C)** | 0.05 | 0.863 | 0.460 | 0.559 |
| **BALGHAT (D)** | 0.05 | 0.168 | 0.155 | 0.161 |

**Table 8: Correlation of arsenic concentration in groundwater in various seasons**

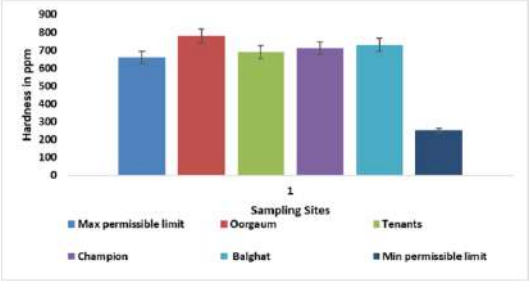
| Seasons during 2014 | | Correlation | Sig. |
|---|---|---|---|
| 1 | Season 1 & Season 2 | 1.000 | .000 |
| 2 | Season 1 & Season 3 | .972 | .028 |
| 3 | Season 1 & Season 4 | .983 | .017 |

**Table 9: Comparison of arsenic concentration in Groundwater in various Seasons**

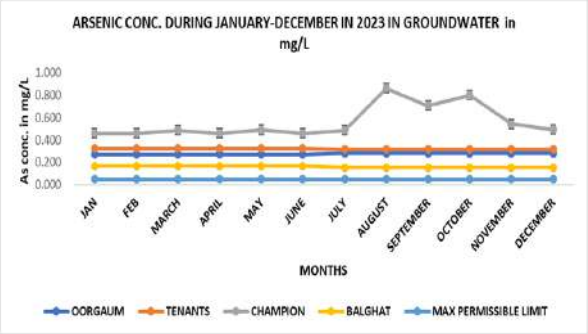
| Seasons | Paired Differences | | | | | t | df | P-value |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | Lower | Upper | | | |
| 1 – 2 | -.000208 | .000417 | .000208 | -.000871 | .000455 | -1.000 | 3 | .391 |
| 1 – 3 | -.052125 | .109947 | .054974 | -.227075 | .122825 | -.948 | 3 | .413 |
| 1 - 4 | -.034000 | .073957 | .036979 | -.151683 | .083683 | -.919 | 3 | .426 |




**Graph 1: Assessment of Arsenic in the soil (mg/kg) in various sampling sites during January-December 2023**




**Graph 2: Average Hardness in groundwater in sampling sites in 2023.**




**Graph 3: Concentration of arsenic in groundwater in mg/L in sampling sites during January-December 2023**

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**RESEARCH ARTICLE**

# Analysis of Virtually Shopping Customer Employing RFM Technique and Clustered using Machine Learning Technique

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## ABSTRACT

Enhancing data gathering and leveraging the acquired data to derive valuable insights is a fundamental responsibility within the domain of customer segmentation. In this study, all relevant criteria are employed to effectively extract information from the available data. To attain the desired results, many techniques are employed, including the utilization of k-means clustering to categorize clients into clusters based on their shared tastes and requirements. The concept of clustering refers to the process of grouping similar data points together based on their inherent the identification of customers that are unlikely to contribute to the business's growth can be achieved by employing the K-means algorithm. Subsequently, the effectiveness of these customer classifications can be assessed through the application of silhouette analysis. The data will undergo normalization using the min-max normalization approach. The main aim of normalization is to determine the likelihood that a specific score will fall within the normal distribution of the dataset. Simultaneously, the process of clustering labeled data will be carried out alongside the clustering of unlabeled data, utilizing the same clustering approach. The data points will be categorized according to the degree of similarity in their feature structures. The application of Silhouette Analysis, a technique that measures the distances between points inside a cluster and facilitates the generation of visual representations for classifying different types of data, will be employed to complete the data analysis. The present technique quantifies the spatial separation among different sites within a given cluster. Furthermore, the RFM methodology was employed to ascertain the worth of individual consumers by analyzing their past purchasing patterns.

**Keywords:** Customer segmentation, K-means Clustering, RFM Analysis, min-max normalization approach, Silhouette Analysis

# INTRODUCTION

The market has witnessed an influx of new enterprises and entrepreneurs, hence intensifying the competition faced by established organizations as they strive to effectively engage and keep clients. According to Bardaki (2018)[1], the preceding development has rendered it suitable for firms of varying scales to prioritize the provision of outstanding customer service. Furthermore, the ability of a corporation to understand the different needs of its consumers and develop tailored approaches to address those needs will lead to enhanced overall customer service. The implementation of a structured client service framework facilitates the acquisition of such valuable insights. Customers in each subgroup share market characteristics (Premkanth, 2012)[2]. Traditional market analytics sometimes struggle to effectively handle a large customer base. However, the emergence of big data concepts and machine learning has led to the use of automated consumer segmentation approaches. In this paper, the utilization of the k-means clustering algorithm is employed (Goyat, 2011)[3].

This research study encompasses a series of sequential steps in its methodology. Furthermore, during the initial phase, the existing data inside the dataset will undergo pre-processing utilizing the Knowledge Discovery technique, with the aim of extracting pertinent information for training purposes through the utilization of a machine learning algorithm. The subsequent procedure involves the implementation of the Min-Max normalization approach. The basic objective of normalization is to determine the probability of a score being within the normal distribution of the data. Simultaneously, the clustering technique is employed on the unlabeled data in order to ascertain groupings within the dataset. The data is categorized into clusters based on their degree of similarity in relation to a specific attribute. In the ultimate phase of the system, Silhouette Analysis will be employed to assess the degree of segregation among data points within a solitary cluster, so enabling the generation of a visual depiction of the model's classification.

**CUSTOMER CLUSTERING**

Companies have had to grow their profitability and company through time as a result of fierce competition in the business field to meet customer expectations and attract new clients depending on their desires. It's tough and time-consuming to identify and respond to each customer's needs. This is owing to the fact that, among other things, clients have a diverse set of aims, interests, and preferences. Customer segmentation, as opposed to a "one-size-fits-all" strategy, divides customers into groups based on comparable characteristics or habits. Customer segmentation is a marketing strategy that divides a market into distinct, homogeneous groups. The data used in the customer segmentation strategy, which divides customers into categories, is based on a number of factors, including regional circumstances, economic patterns, and demographic trends, and behavioral patterns. A client segmentation technique can help a company's marketing resources be better utilized (Hemashree Kilari )(2022)[4].

Managing customers and identifying their likes and dislikes plays a vital role in market business. It has been observed that companies face losses because they are not able to identify the potential customers that will bring them profit. One of the many reasons of these losses are that the companies are using mass marketing tactics that are since whatever we are selling would be liked by everyone. These tactics are time-consuming, expensive, and even proved non-profitable. These strategies are ineffective since every consumer is unique, necessitating the use of some form of algorithm or practice to categories them based on the similarity of their preferences and direct our attention to those groups. To find hidden patterns in data and make future decisions that will be more effective, machine learning is utilized. The hazy idea of which section to target is made clear by the implementation of segmentation. Customer segmentation is the practice of classifying consumers into groups based on similar behavioral patterns and customers into distinct groups based on different behavioral patterns. Suppose a brand focuses on all the customers that are visiting their website but some of them are just browsing their site without intending to buy anything, such people make it difficult for the seller to sell his product as he is targeting everyone. In these types of situation customer segmentation is used (Garima Sharma) (2021)[5].

**MACHINE LEARNING**

We've seen machine learning in action in a variety of businesses, like Facebook, where it helps us identify ourselves and our friends, and YouTube, where it helps us discover new content, where it recommends movies based on our preferences. Machine learning is divided into two types: unsupervised learning and supervised learning. A data analyst often employs supervised learning to address problems like classification and regression, implying that the data in this case is targetable and that we want to anticipate in the future, such as assessing a student's worth or the amount of monthly costs. Unsupervised learning, on the other hand, may or may not have a label or goal in mind. Because it is based on a mathematical model, clustering, for example, does not have a changeable goal. For instance, we might want to group students depending on their learning interests. or product purchases. Strong competition exists in the marketing business, particularly malls, in order to boost consumer numbers and so produce big profits. Many retailers and other marketplaces are already using machine learning to achieve this goal. Malls and shopping centres use the information they collect from customers to construct machine learning models that target the right individuals. This not only boosts revenue and visitor numbers, but it also increases business efficiency [4].

K-means clustering is an unsupervised machine learning approach that segments a dataset into K groups based on shared features. Applying min-max scaling, which converts all the integers to a range from 0 to 1, is one way to normalize a dataset. The clustering precision of the K-means algorithm has been studied in the past, with notable studies including: Mohamad (2013)[6] looked on how normalizing data impacted the accuracy of K-means clustering. In a study comparing K-means' performance on normalized and unnormalized datasets, min-max scaling was found to significantly improve the clustering algorithm's accuracy. Mukhametzyanov (2023)[7] looked into the effect that various normalization techniques had on the accuracy of K-means clustering. Min-max scaling, Z-score normalization, and decimal scaling were all tested to see which would improve K-means' clustering performance the best, and min-max scaling was shown to be the most effective. What factors should be considered while settling on the optimal number of clusters?

Dendrograms are a useful tool for representing the results of a hierarchical clustering investigation. They show how the various data clusters are related to one another and how the data was organized. To cite Forina (2002)[8]. The optimal number of clusters can be identified by analyzing the node distances displayed in the dendrogram. Finding the longest horizontal line that does not intersect any existing clusters is a frequent approach to deciding on the optimal number of clusters. This distance, often known as the "elbow" or "knee" point, measures the optimum number of clusters for the data collection. To help businesses better comprehend their data and make informed decisions about consumer segmentation, they can use dendrograms to visualize the results of hierarchical clustering.
The accuracy of the segmentation model is highly correlated with the number of clusters used in the analysis. There is a danger of oversimplification in segmentation with too few clusters, and of incomprehensibility un segmentation with too many. Silhouette analysis can be used to determine the optimal number of clusters by maximizing similarity within each group and minimizing similarity between groups. Determining the optimal number of clusters can help improve the segmentation model's accuracy and performance. According to Bell (2007)[9], increasing the number of clusters can result in less accurate segmentation models due to the curse of dimensionality. As the number of variables or dimensions in a dataset increases, the complexity of detecting meaningful patterns or clusters also increases. Another study by (Jain, 1988)[10] demonstrated that excessive use of clusters—or "over-segmentation"—can reduce the accuracy of the segmentation model. This occurs when the size of the clusters is too small to give meaningful subsets of the data. However, the accuracy of the segmentation model may degrade if too few clusters are used (under-segmentation). If the clusters are too large, they won't be able to identify smaller variations in the data.

RFM analysis with K-means clustering with hyperparameters provide a more accurate and data-driven technique for consumer segmentation. RFM analysis takes into account the recency, frequency, and monetary value of client purchases, allowing for a deeper understanding of customer behaviors and preferences. K-means clustering with hyperparameters automates the segmentation process, doing away with the requirement for labor-intensive and prone-to-error manual segmentation. A more accurate and efficient technique of customer segmentation is

accomplished by integrating RFM analysis with K-means clustering with hyperparameters. Gupta (2006) [11] compared the efficiency of RFM analysis to that of more conventional approaches like demographic segmentation and behavioral segmentation in predicting client retention and loyalty. The research found that RFM analysis is an effective method for consumer segmentation because it outperformed more traditional methods in gauging client loyalty and retention. Traditional versus hyper parameterized K-means clustering: According to studies (Chu, 2021)[12], K-means clustering with hyperparameters outperforms regular K-means clustering when used to the task of customer segmentation. Hyper parameterized K-means clustering outperformed baseline methods in both effectiveness and stability. The optimal number of clusters was found to be determined by using hyper parameter tuning procedures like the elbow approach and silhouette analysis. Shalabi (2006)[13] A new range of values is found from the old ones by the scaling, mapping, or pre-processing technique known as normalization. Forecasting and prediction can both be done with this strategy. (PATRO, 2006)[14] There are several ways to predict or anticipate the similarities, but each strategy is unique based on the data it receives as input and how it interprets the information. To preserve the large range of variation, normalization techniques must be used to bring the anticipated outcomes closer together.

In order to better understand the need of normalization, let us examine an example. For instance, the dataset for height and weight may contain a range of measurement units, such as meters, inches, and grams. The findings of the data processing were skewed due to the large range of units. Data must be standardized or normalized in order to remove dependency on multiple measuring units and achieve a single measurement unit. The process of normalization involves reducing a large range of data to a single range, usually between -1 and 1, depending on the weight of the data. Among the methods used to normalize data are decimal scaling, min-max normalization, and Z-score normalization. I looked into each strategy and tried to determine its use and limitations for a long period.

## PROPOSED WORK

The process is divided into several discrete steps. Furthermore, as part of the initial phase, the available data inside the dataset will undergo preprocessing using the Knowledge Discovery technique. Subsequently, a machine learning algorithm will be employed to extract the significant data for training purposes. The subsequent stage involves implementing the Min-Max normalization technique. The primary aim of the normalization procedure is to assess the probability of a score being situated within the normal distribution of the dataset. During this process, the clustering technique is employed to analyze the unlabeled data and identify clusters within the dataset. Within this section, the data is categorized into several groups according to the degree of similarity they possess in relation to a specific characteristic. During the final stage of the system, Silhouette Analysis will be employed to assess the proximity of data points within a given cluster. This process enables the creation of a graphical representation that accurately reflects the categorization output produced by the model.

**RFM ANALYSIS FOR THE ANALYSIS OF DATA:**

The RFM (recency, frequency, and monetary) model, proposed by Hughes (1994)[15], is a behaviour-based model that enables the prediction of customers' purchasing patterns. The concept of recency pertains to the temporal proximity of a buy event, while frequency denotes the quantification of purchase occurrences within a specified temporal interval. Monetary, on the other hand, encompasses the aggregate expenditure incurred within the same temporal framework (Wang, 2022)[16]. The utilization of customers' perceptions of the product, brand, perks, and loyalty as a segmentation variable can be facilitated through the aforementioned three behavioural characteristics.

**Use of the RFM Model**

Organizations that adopt the RFM (Recency, Frequency, Monetary) framework observe enhanced response rates, reduced order costs, and increased profitability. In order to apply the RFM model, it is necessary to associate each customer's name and address with a unique identifier, such as an account number, and link it to their respective orders. Additionally, it is crucial to record each transaction in conjunction with the corresponding unique identifier (Hughes, 1994)[15]. In order to ascertain significant and esteemed clients, RFM analysis examines the transaction

history by considering factors such as the timing of purchases, frequency of purchases, and monetary expenditure (Miglautsch, 2002)[17]. The RFM methodology facilitates the categorization of consumers into different segments. The consumer base was categorized into four distinct segments according to their varying degrees of uncertainty: spenders, frequent customers, top customers, and spenders.

**MIN-MAX SCALING**

Normalization can be conceptualized as a technique for scaling, mapping, or pre-processing data. One potential strategy for broadening the scope of the search is to leverage the existing coverage area. Undoubtedly, the utilization of this tool holds significant potential in facilitating the formulation of future projections or predictions. There exist multiple methodologies for generating predictions and forecasts, which may yield disparate outcomes. Normalization is a technique employed to reconcile divergent predictions and forecasts, as noted by Ali (2022)[18].

The Min-Max Normalization technique involves applying a linear modification to the original range of data. The Min-Mix Normalization technique is a strategy that aims to maintain the inherent relationships present in the original dataset. The utilization of Min-Max normalization is a straightforward approach that effectively scales the data to conform inside a predetermined range (Ali, 2022)[18].

According to the Min-Max method of normalizing

$$A' = \left(\frac{A - \text{min value of A}}{\text{max value of A} - \text{min value of A}}\right) \times (D - C) + C$$

Where,

A' contains Min-Max Normalized data one

If pre define boundary is [C, D]

If A is the range of original data

& B is the mapped one data then, (1)

Formulation of Min-Max Normalization (PATRO, 2015)[19]

Normalization, also known as min-max scaling, is a technique utilized to transform numerical data into a standardized range between 0 and 1. When employed alongside k-means clustering, it assists in the process of customer segmentation by enabling more impartial evaluations of features across various units and scales. This literature review investigates the advantages and disadvantages associated with the utilization of min-max scaling in k-means consumer segmentation.

**The utilization of dendrograms for cluster representation:**

A dendrogram is a visual depiction of the outcomes derived from an examination of hierarchical clustering. Chehreghani (2020)[20] asserts that it illustrates the interconnectedness of entities based on the degree of similarity or dissimilarity in their attributes. A dendrogram is a graphical representation that depicts groupings as branches and individual objects as leaves inside a tree-like structure. The initial stage in constructing a dendrogram involves the generation of a distance matrix that quantifies the dissimilarity between each item within the collection. The distance matrix exhibits the proximity or similarity between each pair of elements. Various distance metrics, such as the Euclidean distance or the cosine similarity, might be employed for this objective. The user's text does not contain any information to rewrite. Next, the objects are clustered using the hierarchical clustering technique, which is applied to the distance matrix. The technique begins by considering each object as an own cluster, and subsequently combines them iteratively until only a single cluster remains. The algorithms utilized in hierarchical clustering can be classified into two main categories: agglomerative and divisive.

The determination of the optimal number of clusters for subsequent analysis can be achieved by employing a dendrogram, which provides a graphical depiction of the hierarchical organization of the data. The process of selecting a specific height threshold on the dendrogram and subsequently dividing the tree at that level results in the

determination of the most suitable number of clusters. Subsequently, the created groups are utilized in subsequent investigations or depictions.

**The process of interpreting dendrograms**

The vertical axis of the dendrogram represents the distance or height between groupings. The degree of fusion or merging directly correlates with the dissimilarity of the clusters. The dendrogram's horizontal axis reveals the presence of clusters, whether they consist of objects or individuals. The positioning of the objects in the lower section of the dendrogram can either be assigned randomly or reflect the initial organization of the data.

Identify the points of intersection or fusion of branches within the dendrogram. These dots show clusters that exhibit varied degrees of resemblance. To determine the number of clusters, it is necessary to establish a height threshold on the dendrogram and thereafter partition the tree at that specific level. The clusters that emerge from the application of this criterion to the dataset are the assemblages of items that exhibit the highest degree of similarity to one another. Verifying k-means clustering findings: k-means clustering results can be verified with a dendrogram by comparing the dendrogram's structure to the clusters produced by the algorithm. The validity of the clustering results is further supported if the clusters produced by k-means agree with the dendrogram's structure (Nindhia, 2015)[21].

**K-MEANS CLUSTERING**

K-means clustering is a widely used unsupervised machine learning technique that involves the grouping or clustering of data points according to their similarity. According to Yong (2010)[22], this approach is extensively employed in the fields of data mining, market analysis, and client segmentation. The k-means algorithm partitions data into a predetermined number of clusters, as chosen by the user. Subsequently, the algorithm utilizes the measure of distance between individual data points and the centroid of each cluster to ascertain the appropriate cluster assignment for each data point (Yong, 2010)[22]. The centroid of a cluster is defined as the arithmetic mean of all the data points within the cluster.

The k-means algorithm operates as follows:

I. The initial k centers are randomly selected.
II. The data points are organized into k clusters by assigning them to their respective centroid.
III. Calculate the centroid for each cluster.
IV. Each data point is assigned a new centroid, replacing the old centroid.

Continue to repeat steps 3 and 4 until both the centroids and medians remain within a predetermined threshold, indicating stability in the clustering process. The k-means algorithm generates k clusters, each of which consists of data points that exhibit the highest similarity within their respective cluster and the lowest similarity with data points in other clusters.

**SILHOUETTE ANALYSIS**

Silhouette analysis is employed in unsupervised learning methodologies such as k-means clustering to evaluate the precision of the resultant clusters. The degree of conformity of each data point inside its cluster is assessed by comparing it to the other data points in that cluster. The silhouette score is a metric that measures the quality of clustering results. It is a numerical value that falls within the range of -1 to 1. Higher scores, closer to 1, indicate well-defined clusters, while lower scores, closer to -1, suggest less distinct clusters. This score is utilized to quantify the level of isolation across clusters (Wang, 2017)[23].

**The subsequent sections delineate the many processes involved in silhouette analysis**

The silhouette score for each data point is provided below. It is necessary to calculate the average distance of each data point from all other points inside its own cluster, as well as from all points in the next cluster. The silhouette

score of a point is determined by dividing the difference between the distances to the nearest cluster (b) and the second nearest cluster (a) by the greatest value between a and b. Analyze and interpret the results: If a cluster exhibits a high average silhouette score, it indicates that the data points within the cluster are very similar to each other and are distinct from the data points in other clusters. A cluster exhibiting a low average silhouette score indicates inadequate isolation from adjacent clusters and is likely to have data points that exhibit limited comparability among themselves.

The application of silhouette analysis enables the evaluation of clustering outcomes in terms of their accuracy and facilitates the identification of the optimal approach for partitioning a given dataset into distinct groups. In order to ascertain the relevance and utility of the created clusters for subsequent study, it is feasible to ascertain the optimal number of clusters by means of comparing the silhouette scores across different cluster quantities (Wang, 2022)[16].

## RESULT AND DISCUSSION

The entire process is strategically divided into distinct phases to optimize the utilization of data for effective machine learning training.

**Pre-processing and Knowledge Discovery**

The initial phase focuses on pre-processing the existing dataset through the Knowledge Discovery process. This involves extracting pertinent information that is crucial for training machine learning algorithms. The aim is to filter and refine the dataset, ensuring that only the most relevant data is considered for further analysis.

**Normalization using Min-Max Normalization**

Following the pre-processing phase, the dataset undergoes normalization using the Min-Max normalization technique. This step is pivotal in determining the probability of a score occurring within the normal distribution of the data. By standardizing the data range, the normalization process enhances the algorithm's ability to uncover patterns and relationships within the dataset.

**Clustering Technique for Unlabeled Data**

Simultaneously, an advanced clustering technique is applied to the unlabeled data to identify inherent groups within the dataset. Data points are clustered based on the similarity of their features, allowing for the discovery of underlying patterns and structures. This step facilitates a more nuanced understanding of the relationships among data points.

**Silhouette Analysis for Data Interpretation**

In the final phase, a sophisticated Silhouette Analysis is employed to measure the distance between different points within a cluster. This analysis provides a graphical representation of the classification between various data points, allowing for a visual interpretation of the model's performance. Silhouette Analysis aids in assessing the effectiveness of the clustering algorithm and highlights the distinctiveness of clusters.

**Data Analysis and Pre-Processing**

Dataset: https://archive.ics.uci.edu/dataset/352/online+retail. In order to perform an exhaustive examination of the customer data, we employ a variety of robust data science libraries, such as Matplotlib, NumPy, and Pandas. These libraries are of the utmost importance for perusing, manipulating, and visualizing the available data in an efficient manner; they facilitate insightful observations and well-informed decisions.

**DATASET FEATURES**

**ID of the Customer:** Serves as a unique identifier for each customer, allowing for individual tracking and analysis.

**Invoices of the Customer:** Records the number of invoices associated with each customer, providing insights into their transactional activity.

**Unit Price of Product:** Reflects the cost per unit of the product purchased, aiding in understanding the pricing dynamics and customer spending patterns.

**Quantity:** Represents the quantity of products bought by each customer, contributing to a comprehensive assessment of purchasing behavior.

**Invoice Date:** Captures the date of each invoice, facilitating temporal analysis and enabling the identification of trends over time.

**Amount:** Quantifies the monetary value associated with each transaction, offering a holistic view of customer expenditure.

**ANALYSIS PROCESS**

The analysis process involves importing the dataset using Pandas, utilizing NumPy for numerical operations, and employing Matplotlib for creating visualizations. Through these libraries, we aim to uncover meaningful insights into customer segmentation, transaction patterns, and overall trends within the dataset. By combining the features of the customer data with the capabilities of these data science libraries, the analysis becomes a robust and systematic exploration, leading to a richer understanding of the dynamics and behaviors inherent in the customer dataset From above Table 1 and Fig 3 the analysis indicates that the dataset holds a wealth of diverse and valuable information, providing numerous opportunities for extraction and exploration. With a comprehensive set of features such as customer demographics, product details, and transaction history, the dataset opens up a broad spectrum of analytical possibilities. It allows for in-depth examinations of customer behavior, product popularity, purchasing trends over time, and the identification of correlations between various variables. The dataset's richness also facilitates segmentation of customers into distinct groups, enabling targeted marketing and personalized strategies. Furthermore, the presence of patterns and trends within the data supports predictive modeling, aiding in forecasting future trends, customer preferences, and market shifts. Businesses can leverage this variability to optimize their operations, improve efficiency, and stay competitive. Continuous analysis and extraction of insights from this diverse dataset contribute to ongoing efforts in adapting to changing market conditions and enhancing overall business performance. In summary, the dataset presents a robust foundation for comprehensive exploration and extraction of valuable insights that can drive strategic decision-making and business success.

**RFM Analysis**

The RFM (Recency, Frequency, Monetary) model serves as a powerful technique in understanding and quantifying customer behavior based on their responsiveness to transactions. This methodology involves the assessment of three key parameters

**Recency:** The Recency parameter delineates the recentness of a customer's purchase activity. It aims to identify when a customer last engaged in a transaction, providing insights into their ongoing connection with the organization. The calculation of Recency involves assigning values based on the most recent purchase date, forming a foundation for subsequent reminders and targeted marketing campaigns.

**Frequency:** Frequency relates to how frequently a customer engages in transactions. This parameter considers factors such as product type, pricing, and customer behavior regarding product replacements. By analyzing Frequency, organizations can manage marketing costs and efforts more effectively, attracting new customers and retaining the loyalty of existing ones.

**Monetary:** The Monetary parameter helps identify the amount of money a customer spends on purchases and reveals the spending behavior of different customer segments. Calculated using a specific syntax, Monetary value aids in understanding the nature of customer expenditure, providing valuable insights for targeted marketing and tailored business strategies.

From above Table 2 In the initial steps of RFM analysis, customers are assigned values for Recency, Frequency, and Monetary parameters. Subsequently, the customer list is segmented into tiers based on these dimensions (R, F, and M), forming the basis for targeted marketing and advertising campaigns. This sophisticated approach allows organizations to tailor their strategies effectively, optimizing customer engagement and maximizing business impact.

Fig 4 by visualizing the distribution of clients across various recency, frequency, and monetary divisions, this graph helps firms adjust their marketing tactics and offerings. Shorter bars represent fewer clients, whereas higher bars represent a greater amount of customers falling into that group. By looking at how the bars are distributed throughout the Recency, Frequency, and Monetary categories. For example: A large proportion of clients in the "0-50 days" recency category points to a robust recent clientele. More consumers in the higher frequency categories point to a devoted following of buyers who make up to four purchases annually. Higher bars in the Monetary "High spenders" category denote important consumers that make a considerable revenue contribution up to a minimum purchase of $50.

**Building Dendrogram**

A dendrogram is a diagram that illustrates the distances between attributes in sequentially merged classes. To prevent overlapping lines in the diagram, it arranges the merged pairs of classes adjacent to their neighbors. The information within this diagram is subsequently utilized for clustering algorithms. In essence, a dendrogram provides a visual representation of the relationships and distances between paired classes, facilitating a clearer understanding of clustering patterns and aiding in the analysis of hierarchical structures. From Above Fig 5 upon careful inspection of the dendrogram, it is observed that there are discernible points where the vertical lines display notable shifts in height. These points may suggest potential cluster boundaries, and the number of these significant changes could correspond to the optimal number of clusters.

**Specific Findings:**

**Three Potential Clusters:** A distinct change in height is noticed, indicative of a potential split into three clusters. The branches below this point represent relatively homogeneous groups of data points.

**Four Potential Clusters:** Another notable change in height occurs, suggesting a possible alternative clustering scenario with four distinct groups. This is marked by a significant divergence in the dendrogram structure.

**Decision-Making:** The choice between three or four clusters depends on the specific characteristics of the dataset and the objectives of the analysis. It may be beneficial to experiment with both scenarios and evaluate the performance of clustering algorithms to determine the most suitable number of clusters for extracting meaningful insights from the data.

**Model Building Using K-Means Clustering**

The K-Means clustering algorithm is a versatile tool for partitioning data into distinct groups based on similarity. When utilizing the K-Means class, several parameters contribute to the customization of the algorithm. In this particular implementation, the focus is on three key parameters:

**Init (Initialization Method):**

Init defines the method for initializing centroids, which are crucial in determining the cluster centers. The 'k-means++' option, chosen here, employs a smart strategy to place the initial centroids, enhancing the convergence speed of the K-Means algorithm.

**n_clusters (Number of Clusters)**

The n_clusters parameter sets the number of clusters the algorithm aims to form. In this case, the value is set to 3, indicating the intention to identify three distinct groups within the data. Adjusting this parameter allows for flexibility in capturing different levels of granularity in the clustering.

**n_init (Number of Initializations)**

n_init specifies how many times the K-Means algorithm will run with different initial centroid seeds. The algorithm calculates the inertia for each run, and the configuration yielding the lowest inertia is considered the final result. In this implementation, a common practice is to set n_init to 12, ensuring robustness in capturing the optimal clustering outcome.

**Visualization of Clusters**

Above Fig 6 the visual representation of customer clusters reveals compelling insights. Cluster 1 encapsulates customers sharing notable similarities in characteristics and behaviors, presenting a lucrative target for focused marketing campaigns. The concentrated nature of this cluster suggests that tailoring strategies specifically for these customers could yield impactful results. Conversely, Cluster 3, while smaller in size, represents a distinct customer segment. Recognizing this smaller group's unique attributes and preferences is an opportunity for businesses to strategize on expanding and retaining customers within this cluster. By implementing targeted initiatives, businesses can aim to increase the size and influence of Cluster 3. Visual aids, such as bar graphs, prove invaluable in this context. These tools facilitate a quick and comprehensive understanding of customer segments. Bar graphs, for instance, enable businesses to visually discern the composition of each cluster, empowering them to fine-tune marketing efforts with precision. Leveraging such visual aids enhances the effectiveness of marketing strategies, maximizing engagement and ultimately driving revenue.

In essence, the visualization serves as a strategic guide, enabling businesses to navigate customer segmentation effectively and optimize their marketing approach for each distinct cluster.

**SILHOUETTE ANALYSIS FOR OPTIMAL CLUSTERING**

Silhouette analysis is a crucial technique for validating clustering groups and determining the most suitable cluster configuration. The silhouette() function plays a pivotal role in this process by computing the average width of silhouettes. This width serves as a valuable metric for identifying the optimal cluster size. The silhouette average width is plotted against the number of cluster centers to visually identify the optimal cluster configuration. The cluster with the largest silhouette average width is considered the best choice. In this example, the general value of the average width of the silhouette is found to be 3 clusters per the analysis. Upon evaluating the clustering analysis, K-means clustering with Silhouette evaluation method demonstrates superior performance, particularly with k=4. In contrast, hierarchical clustering with the elbow method encounters challenges as it does not perform optimally with the same k value, highlighting the potential ambiguity associated with this approach.

In order to find the ideal number of clusters in a dataset, the Silhouette Analysis graph is a useful tool for cluster analysis. It computes the Silhouette Score for each data point and then averages these values to get an overall score for the clustering configuration.

**X-axis: Number of Clusters (k):**

The number of clusters being assessed in the dataset is shown on the x-axis. It varies from two to fourteen clusters in this instance.

**Y-axis: Silhouette Score:**

The y-axis displays the Silhouette Score, a metric that quantifies an object's cohesiveness (similarity to its own cluster) in relation to its separation (difference from other clusters). The range of the Silhouette Score is -1 to 1. When an item has a high silhouette score, it means that it matches well with its own cluster and poorly with nearby clusters. This is advantageous as it implies a distinct division between clusters. Finding the number of clusters (k) where the Silhouette Score is maximized—a sign of the ideal clustering arrangement—is the aim. The fact that your dataset is divided into three clusters yields the highest average similarity within clusters and the largest dissimilarity across clusters when compared to alternative cluster configurations, as indicated by the higher score in three clusters. From

above Table 3 Each client's cluster assignment is listed in the cluster column, with values of 1 through 3 designating which cluster the customer belongs to. It is anticipated that customers belonging to the same cluster will display comparable behavior or attributes, depending on the clustering parameters (Recency, Frequency, Monetary). Customers in Cluster 1 may, therefore, be people who have recently made small-scale, infrequent transactions. Consumers in Cluster 2 may be those who make regular purchases of moderate value, while consumers in Cluster 3 might be high-value individuals who make regular purchases of large value. You may better satisfy the demands of each segment by customizing your marketing strategy, product offers, and customer service activities to each cluster, which represents a specific portion of your client base with unique behavior patterns.

**COMPARISON OF EXISTING AND PROPOSED SYSTEM**

In Existing system Agglomerative Clustering was used with RFM analysis, after finding the clustering using different k. The greater silhouette score found in 3 clusters same as in k-means. From Fig 8 and Table 4 the proposed technique has significantly improved customer segmentation compared to the existing agglomerative clustering method. With agglomerative clustering, the silhouette score stood at 0.42 when utilizing 3 clusters. However, upon implementing K-means clustering with silhouette analysis, the silhouette score notably increased to 0.46 with the same number of clusters. This enhancement signifies that the proposed technique not only provides a more objective measure for determining the optimal number of clusters but also yields more distinct and well-separated clusters, thus leading to a more refined and effective segmentation of customers.

## CONCLUSION

In the domain of consumer segmentation, enhancing data collection and utilizing the acquired data to derive pertinent insights constitutes a highly consequential undertaking. The utilization of the k-means algorithm the topic of interest is clustering. The application of silhouette analysis is employed to assess the precision of customer classification, while the utilization of K-means aids in the identification and removal of potential clients who are unlikely to contribute to the company's growth. The customer segmentation method involved the application of three approaches: k-means clustering, silhouette analysis, and RFM analysis. One of the most significant findings is that clients who have been active recently have the greatest evaluations in terms of frequency and monetary value. The evaluation of clustering quality is conducted by the application of the silhouette approach, which entails the assessment of both intra-cluster similarity and inter-cluster dissimilarity. The silhouette score is a metric that measures the quality of clustering results. The utilization of three clusters might facilitate firms in customizing their marketing tactics to distinct client segments characterized by comparable demands and preferences, thereby enhancing customer happiness and augmenting income.

## ACKNOWLEDGEMENT

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**Table 1 Feature Analysis**

| Data columns (total 8 columns): | | | | |
|---|---|---|---|---|
| # | Column | Non-Null | Count | Dtype |
| 0 | InvoiceNo | 354321 | non-null | object |
| 1 | StockCode | 354321 | non-null | object |
| 2 | Description | 354321 | non-null | object |
| 3 | Quantity | 354321 | non-null | int64 |
| 4 | InvoiceDate | 354321 | non-null | datetime64[ns] |
| 5 | UnitPrice | 354321 | non-null | float64 |
| 6 | CustomerID | 354321 | non-null | object |
| 7 | Country | 354321 | non-null | object |
| 8 | Amount | 354321 | non-null | float64 |

**Table 2. Result for Recency,Frequency and Monetary Calculation**

| [1] | [2] **CustomerID** | [3] **Recency** | [4] **Frequency** | [5] **Monetary** |
|---|---|---|---|---|
| [6] 0 | [7] 12346 | [8] 325 | [9] 1 | [10] 77183.6 |
| [11] 1 | [12] 12747 | [13] 2 | [14] 11 | [15] 689.49 |
| [16] 2 | [17] 12748 | [18] 0 | [19] 209 | [20] 3841.31 |
| [21] 3 | [22] 12749 | [23] 3 | [24] 5 | [25] 98.35 |
| [26] …… | [27] …. | [28] ……. | [29] …… | [30] ……. |
| [31] 3916 | [32] 18281 | [33] 180 | [34] 1 | [35] 5.04 |
| [36] 3917 | [37] 18282 | [38] 7 | [39] 2 | [40] 38.25 |
| [41] 3918 | [42] 18283 | [43] 3 | [44] 16 | [45] 66.75 |
| [46] 3919 | [47] 18287 | [48] 42 | [49] 3 | [50] 80.4 |

**Table 3. Customer Segmentation using Clusters**

| [51] | [52] **Customer ID** | [53] **Recency** | [54] **Frequency** | [55] **Monetary** | [56] **R** | [57] **F** | [58] **M** | [59] **Cluster** |
|---|---|---|---|---|---|---|---|---|
| [60] 0 | [61] 12749 | [62] 4 | [63] 5 | [64] 98.35 | [65] 0.009119 | [66] 0.4 | [67] 0.430253 | [68] 2 |
| [69] 1 | [70] 12820 | [71] 4 | [72] 4 | [73] 58.2 | [74] 0.009119 | [75] 0.3 | [76] 0.253909 | [77] 1 |
| [78] 2 | [79] 12821 | [80] 215 | [81] 1 | [82] 19.92 | [83] 0.650456 | [84] 0 | [85] 0.085778 | [86] 3 |
| [87] …. | [88] …… | [89] ……. | [90] ……. | [91] ….. | [92] … | [93] … | [94] …… | [95] …. |
| [96] 3191 | [97] 18281 | [98] 181 | [99] 1 | [100]5.04 | [101]0.547112 | [102]0 | [103]0.020423 | [104]3 |
| [105]3192 | [106]18282 | [107]8 | [108]2 | [109]38.25 | [110]0.021277 | [111]0.1 | [112]0.166286 | [113]1 |
| [114]3193 | [115]18287 | [116]43 | [117]3 | [118]80.4 | [119]0.12766 | [120]0.2 | [121]0.351414 | [122]1 |

**Table 4. Comparison of two algorithm**

| [123]**Algorithm** | [124]Agglomerative | [125]K-means |
|---|---|---|
| [126]**Silhouette Score** | [127]0.42 | [128]0.46 |

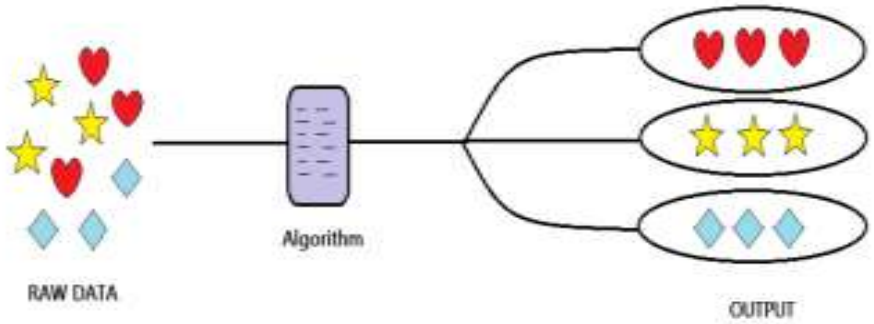
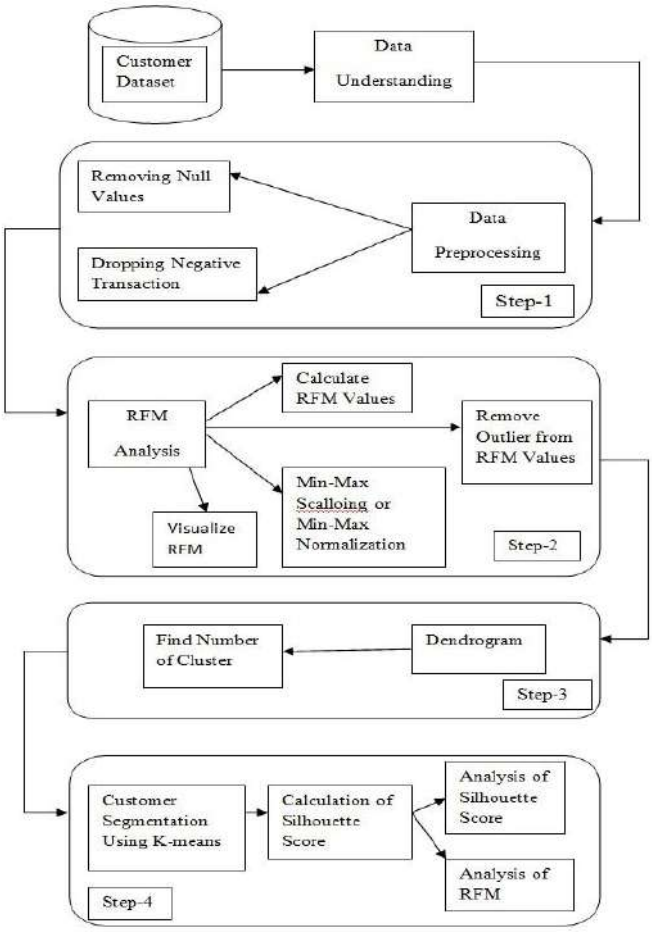



**Fig. 1 Clustering [4]**




[129]

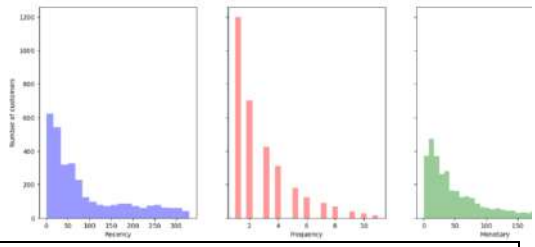
[130]**Fig. 2 Workflow block diagram for Proposed Methodology**

```
Summary..
Number of invoices:  16646
Number of products bought:  3645
Number of customers: 3920
Percentage of customers NA:  0.0 %
Average quantity of product purchased by a customer:  1086.0
Average revenue generated per customer:  1864.39
Average product quantity sold per transaction:  12.0
Average revenue generated per transaction:  20.63
```

[131]

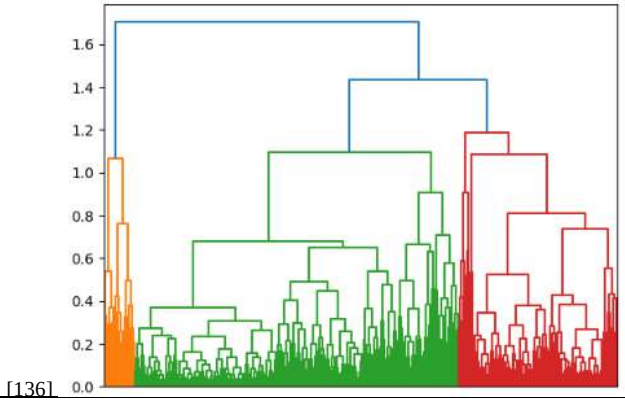
[134]**Fig. 3 Data Analysis of each Attribute**

[132]



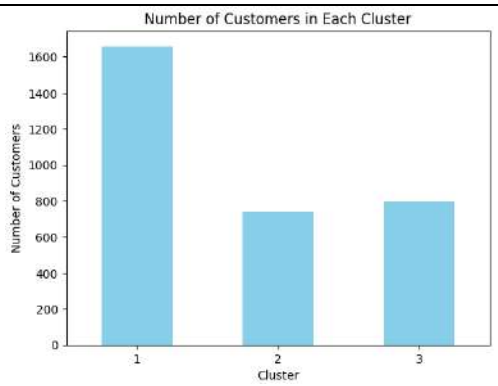
[133]

[135]**Fig. 4 RFM Visualization**



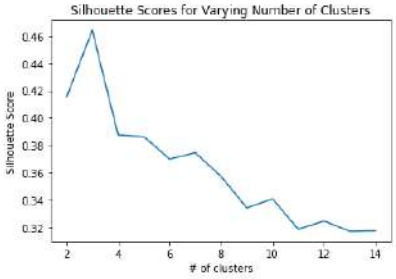

[136]

[138]**Fig. 5 Clusters Representation using dendrogram**



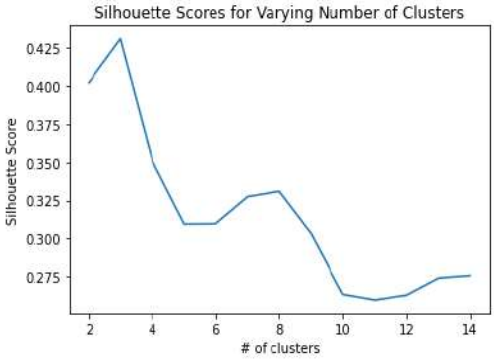

[137]

[139]**Fig.6 Finding Respective Cluster Segmentation w.r.t RFM values**




[140]

[142]**Fig. 7 Clusters found using Silhouette Analysis**




[141]

[143]**Fig.8 Agglomerative silhouette Results**

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**RESEARCH ARTICLE**

# Navigating Fragmentation Maze: Unraveling the Impact of Content Fragmentation on OTT Consumption Habit

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## ABSTRACT

The proliferation of OTT (Over-The-Top) platforms, advancements in mobile technology, improved streaming quality, and the democratization of content creation all have contributed to a transformative media landscape. In this backdrop the current study is an attempt to investigate how far content fragmentation propel the OTT consumption in the media industry. Moreover, it tries to understand customers' preference for OTT platforms compared to traditional platforms based on content fragmentation. As per the previous studies the usage of OTT platforms has tremendously increased during Covid-19 and the content fragmentation is noticed as a new marketing strategy for attracting media consumers. Considering these aspects, present study tries to examine whether this content fragmentation really fuel the OTT viewing habit. Data collected from 100 respondents are analysed using multiple regression and percentage analysis to fulfil the objectives. The results suggest that various benefits and features of content fragmentation like easy availability, easy discovery, user-friendliness, smooth flow, and easy accessibility of contentshave significant impact on OTT consumption habit. In the same way,young media consumers are more interested in OTT platform than traditional channel for video consumption. Thus, this study is very helpful for broadcasters to frame more tactical marketing ideas to attract and maintain customers.

**Key words**: Content fragmentation, OTT platform,Media Consumption, Technology.

## INTRODUCTION

The technological advancements, coupled with the paralyzing effects of the COVID-19 pandemic, have brought about significant configurations in the landscape of media consumption in recent years (Sharma &Lulandala, 2023). The proliferation of digital platforms, the advent of cheap and high-speed internet, and the availability of High-Definition (HD) and Ultra HD (4K) resolutions, along with advancements in audio technologies such as Dolby Atmos, have revolutionized how people access and interact with media content (Mairaru et al., 2019). Moreover, the evolution of mobile technology has also played a crucial role in shaping media consumption patterns (Singh, 2019). With the widespread adoption of smartphones and tablets, consumers can now carry portable devices capable of streaming videos, listening to music, and accessing various forms of digital content (Ernst & Young, 2016). The convenience of mobile devices, coupled with improved internet connectivity, has made media consumption more accessible and seamless, empowering users to enjoy their favourite content at anytime and anywhere (Ulin, 2013; Bentley et al., 2019).

In connection with technology-enabled media consumption, one of the most notable advancements is the rise of Over-The-Top (OTT) platforms. OTT technology enables content owners to manage the process of delivering video content across multiple devices using the internet. Remarkably, amidst the challenges brought about by the pandemic, the OTT industry has emerged as one of the rare beneficiaries (www.argoid.ai). The shutdown of production houses and cinema halls across the nation has created a huge demand for OTT video streaming platforms, offering original series and movies (Shaikh and Aggarwal, 2020; PwC, 2020). In contrast to traditional networks, OTT services like Netflix, Amazon Prime Video, Hotstar, Hulu, and Voot deliver streaming media directly to consumers at their request via the internet, bypassing traditional cable or satellite providers (Purdy, 2018). This shift has enabled users to enjoy on-demand access to a vast array of movies, web series, TV shows, documentaries, and other forms of content, allowing them to personalize their viewing experience according to their preferences and schedules (Puthiyakath& Goswami, 2021). With more and more people subscribing to several OTT platforms, it is no surprise that streaming services are major contributors to the media industry. According to a report published in Economic Times in April 2020, OTT video streaming platforms in India saw a growth of 34 percent in March 2020. Industry experts claim that the current rise in OTT video streaming platform viewing has already fuelled the expansion of these platforms over the next five years. It has been forecasted that OTT platforms' revenue could reach US$4 billion by the year 2025 (www.ibef.org).

At the same time, as we step into 2023, the realm of OTT (Over-The-Top) platforms finds itself standing at a pivotal juncture, poised for significant transformations and advancements. Alongside the growth in OTT platforms, content fragmentation has become a significant challenge for this industry. Content fragmentation refers to the distribution of media content across multiple platforms, creating a fragmented landscape where consumers need to subscribe to multiple services to access their desired content(Deloitte, 2021). This fragmentation arises due to various factors, including the entrance of new players into the market, the rise of exclusive content licensing deals, and the strategic decisions made by content creators and distributors. The impact of content fragmentation on OTT consumption is multifaceted. On one hand, consumers are faced with an abundance of choices and a diverse range of content options. This offers viewers the flexibility to select content that aligns with their preferences and interests. However, on the other hand, the increasing number of subscription-based platforms and the fragmentation of content can lead to subscription fatigue and higher costs for consumers. This maze-like condition can be particularly challenging for viewers who are interested in accessing specific shows or movies that are exclusive to certain platforms.

Anyway, users like to see tailored content based on their previous experiences that may break their preconceived ideas about what to watch. In the most advanced way, content fragmentation offers a clear, simple approach to locate and consume content that is both comfortable and interesting (Begum, 2018; Haryoto, 2015). Many previous studies have examined drivers of high dependency on second screens and continued use of OTT platform, especially during Covid-19 pandemic (Valecha & Jaggi,2020; Gupta &Singharia, 2021). There are studies discussing factors contributing

to the satisfaction and dissatisfactionof OTT services also (Shin & Park, 2021).So, a gap is insinuated that the studies conducted to date have not yet seriously taken into consideration consumers' usage of OTT platforms as a result of content fragmentation. Therefore, this research paper aims to decipher an understanding about the effect of content fragmentation that stimulates the usage of OTT video streaming platforms, among the young consumers. Moreover, this research paper aims to gain a better understanding of the preference for OTT platforms and traditional platforms grounded on content fragmentation.

**Significance of the Study**

The study discussing the effect of content fragmentation on OTT watching habit is useful both to OTT users and broadcasters. To the viewers the viewing experience feels almost personalized when they open their favourite streaming platform and finds a collection of content that is almost entirely in line with their preferences and interests. Study on content fragmentation boost the broadcasters to build loyal audiences and communities around their brands. Moreover, in this period of content overload consumers are confused to understand which one is more important and needed along with subscription fatigue (McAdams, 2019). Therefore, they have to use content fragmentation, a new advancement in technology, which has brought some tremendous changes in the way of enjoying the media contents.

**Literature Review and Conceptual Development**

The existing literature on OTT platforms suggests that the presence of intriguing content is crucial in retaining viewers in an OTT streaming platform and the success of an OTT platform largely depends on its ability to facilitate easy content discovery. The following section will discuss reviews about content fragmentation and OTT platform.

**Content Fragmentation**

Content fragmentation is a buzzword in today's OTT market place. It is a strategy that helps broadcasters to reach niche audiences. The term content fragmentation implies the collection of content that is driven by preferences, tastes, gender and other factors of a specific target group. If the viewers have felt content as relevant and engaging, it will connect the target audience and subscribers remain active (www.muvi.com). Increased content consumption through digital media is changing consumer preferences and attitudes and this development may be ascribed to better internet access, digital gadgets, competitive data pricing in India and the mobile aspect of online media (Bhavsar, 2018). Content fragmentation offers a simple approach to locate and consume content with ease and interest. Distributing different types of content on different Video-On-Demand (VOD) platforms is one of the effects of this approach. The purpose of this trend is to create a variety of platforms that are focused on a specific type of video contents to give the users exactly what they are looking for. It is being adopted in the new broadcasting sector and OTT players are tapping into its many benefits.

**Over-the-Top (OTT)Consumption**

OTT or Over-the-Top refers to streaming services that allow content to be delivered over the internet. As the name implies, the service is offered "on the top" of another platform. It bypasses the traditional satellite cable and broadcasting platforms (Puthiyakath& Goswami, 2021). OTT delivers television video and audio through the internet instead of conventional radio frequencies. This means that OTT platforms do not have to send the same show to everyone at the same time. Content is provided at the request of the consumers to meet his/her requirements. This is the main feature of OTT platforms that differentiates itself from traditional television services.OTT services and television networks can both coexist in the same market. However, it is anticipated that, younger generation will move on to OTT platform, while the older people assumed to prefer traditional television (Negi, 2022).Baccarne, Evens, and Schuurman, (2013) examined in their study, how the emergence of OTT platforms has resulted in the loosing of demand and monopoly of traditional TV and as per their report, the reason for the success of OTT is itscontent quality, removal of time restrictions, usability, flexibility, tailored watching experiences etc., (Sing, 2019).Among the factors the availability of 'personalised content' and effectiveness of 'dubbing' and 'subtitles' for foreign content also noticed as the reason for the increased OTT consumption (Begum, 2018). Along with the above-mentioned variables, accessibility of user-friendlycontent at lower prices makes it more attractive and it leads to

binge watching habit among the youngsters (Sujatha et al., 2015; Matrix, 2014). At the same time, the KPMG's annual cable &OTT report shows that, though the cost is a matter of consideration when buying OTT services, content still influences the majority of customer decisions (Purdy, 2018). Therefore, the acceptance and adoption of OTT depends on perceived risk, trust, age and many factors that are identified in many studies (Chang & Chen, 2018; Dasgupta, & Grover, 2019).

**Objectives**

To analyse the influence of content fragmentation on OTT consumption habit.

**Hypotheses**

The content fragmentation has significant impact on the OTT consumption habit.

**Conceptual Model**

Fig.1.Initial model

**Research Design**

This study used a survey methodto collect theprimary data from young respondents belonging to the age between 18-35 years. A non-probability-purposive sampling method has been adopted to select the samples from South India, specifically from the State of Kerala. Out of 116 respondents approached, 100 responses were found as filled and these were utilized for statistical analysis and interpretation. The data were collected during the period of July 2023 to Oct 2023 and a pilot study of 30 respondents were carried out. Based on the inputs from pilot study, the statements measuring content fragmentation were redrafted on a Likert scale and asked to give their degree of agreements regarding the its features infive-point scale ranging '1' for 'strongly Agree' to 5 'strongly Disagree'. As the variables measuring OTT Consumption habit consist of five variables no factor analysis is done for variable reduction. To determine the preference for OTT platforms over traditional platforms,based on content fragmentation, respondentias are asked tick either in 'Traditional Platform' or in 'OTT Platform' after going through the statements.

**Data Analysis**

The collected dataare analysed with the help ofsoftware, SPSS-20 and Excel.Before moving on to the detailed analysis, researcher checked the reliability of the content fragmentation variables namely easy availability, easy discovery, user-friendliness, smooth flow and easy accessibility of content and found that as .892 (Table 1). Then to study the influence and impact of content fragmentationon OTT consumption habit, 'multiple regression' analysis has been used (given in Table-2) and to assess the preference for OTT platforms or traditional channels in terms of content fragmentation features, percentage analysis has been applied and the results are shown in Table 3.

**Regression Equation:** Consumption Habit = .419 (Constant)+.145 (Easy availability of content) +.586 (Easy discovery of content) + .058 (User-friendliness of content) + .322(Smooth flow of content) + .045 (Easy accessibility of content).

As per the regression equation, for every one unit of increase in the easy availability of content, OTT consumption get increased by .145 i.e., 14.5%. For every one unit of increase in the easy discovery of content, OTT consumption get increased by .586 i.e., 58.6%. Likewise, for every one unit of increase in the user-friendliness of content leads to .058 i.e., 5.8%. of OTT consumption. The one unit increase in the customers' perception regarding smooth flow of content result in the .322 (32.2%) growth in OTT consumption habit. Finally, an increase in the feeling of easy accessibility of content will result in .045 or 4.5% impact on OTT consumption.

**$H_1$: The content fragmentation has significant impact on the OTT consumption habit.**

As per the Table2.a, the adjusted $R^2$ value is .926 and this depicts that, 92.6% of the OTT platform viewing habit is determined by the identified variables of this research, describing the OTT platform viewers' perceptions about content fragmentation. To statistically test the above hypothesis (H1), five sub hypotheses are framed and multiple regression results of these are discussed below.

**$H_{1a}$: Easy availability of content has significant impact on the OTT consumption habit.**

$$\text{Model I: } Y_1 = \alpha + \beta X_1 + e$$

As per the study the feature of content fragmentation i.e., easy availability of content positively influences the OTT plat form consumption of young viewers belonging to the age group of 18-35 and this effect (β=.145) is significant ( p≤05) too.

**$H_{1b}$ Easy discovery of content has significant impact on the OTT consumption habit.**

$$\text{Model II: } Y_2 = \alpha + \beta X_2 + e$$

As per the study the feature of content fragmentation i.e., easy discovery of content also noticed with positive influence on the OTT platform consumption and its effect is .586. As the p value is less than .05, this influence is significant.

**$H_{1c}$ User-friendliness of content has significant impact on the OTT consumption habit.**

$$\text{Model III: } Y_3 = \alpha + \beta X_3 + e$$

Another feature tested isuser-friendliness of content and it resulted withsignificant positive impacton the OTT plat form usage (β=.058, p≤05).Thus, like easy availability and easy discovery, the feature of user-friendliness of content fragmentation contributes to OTT consumption.

**$H_{1d}$: Smooth flow of content has significant impact on the OTT consumption habit**

$$\text{Model IV: } Y_4 = \alpha + \beta X_4 + e$$

Again, as per the study the feature of content fragmentation i.e., Smooth flow of content is positively influencing the OTT platform consumption of youth and this effect is highly significant ( β=.322, p≤.001) too.

**$H_{1e}$Easy accessibility of content has significant impact on theOTT consumption habit.**

$$\text{Model V: } Y_5 = \alpha + \beta X_5 + e$$

In the case of easy accessibility, as per the test, customers responses are positively influencing OTT consumption and it is also significant.

## DISCUSSION

The major focus of this study was to identify the impact of content fragmentation on OTT consumption pattern and here, the variables identified to determine the reasons for OTT consumption habit are found in line with the existing studies(Menon, 2022; Sadana& Sharma2021).As per the results of this study, the variables studied to measure the perceptions propelling content fragmentation have significant influence (availability -β= .145, t=2.54 p=.012; discovery- β =.586, t=8.788, p<.000; user-friendliness-β=.058 , t=1.65 p=.002; smooth flow-β=.322, t=6.636, p<.000and accessibility- β= .045 , t=1.552 p=.024)on the usage of OTT platforms also.

The results of the present study echo the contributions of existing studies, emphasizing that content, being the core element of entertainment, should be made available in the right manner, making the consumption more exciting(Matrix, 2014). However, despite of having its own challenges (Rojaset al., 2020)content fragmentation has become quite common among OTT platform users and content fragmentation creates a personalized viewing experience for the target audiences (Podara et al., 2021). This new trend of content fragmentation has its own importance in this era where people are facing the situation of content overload (Sivamol& Suresh, 2019). The content

fragmentation makes it easy for the users to access their most needed content. But, the limitation of big screen show, subscription costand risk perceptions are to be addressed seriously.

## CONCLUSION

Content fragmentation in the OTT industry presents both opportunities and challenges. While consumers benefit from a wide variety of content choices and personalization options, they also face the complexities of managing multiple subscriptions and platforms. Content creators and distributors must navigate the competitive landscape while striving to maximize their reach and revenue. Besides, customer centric content play a very significant role in shifting the consumers from television to OTT platforms and the personalized contents and convenience in using contribute in escalating the consumers shift towards OTT platforms (Chen.Y.N.K, 2019).

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**Table 1 Reliability Statistics**

| Cronbach's Alpha | N of Items |
|---|---|
| .892 | 5 |

Source: Primary data Analysis

**Table 2: Multiple regression Analysis for content fragmentation perceptions and OTT consumption habit.**

**Table 2.a Model Summery**

| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | .964[a] | .929 | .926 | .26112 |

Source: Primary data Analysis

a.Predictors: (Constant), Easy availability of content, Easy discovery of content, User-friendliness of content, Smooth flow of content, Easy accessibility of content.

**Table 2.b ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 84.341 | 5 | 16.868 | 247.387 | .000[b] |
| | Residual | 6.409 | 94 | .068 | | |
| | Total | 90.750 | 99 | | | |

a. Dependent Variable: OTT consumption Habit

b. Predictors: (Constant), Easy availability of content, Easy discovery of content, User-friendliness of content, Smooth flow of content, Easy accessibility of content.

**Table 2.c Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .419 | .121 | | 3.456 | .001 |
| | Easy availability of content | .188 | .074 | .145 | 2.548 | .012 |
| | Easy discovery of content | .654 | .074 | .586 | 8.788 | .000 |
| | User-friendliness of content | .060 | .037 | .058 | 1.654 | .002 |
| | Smooth flow of content | .326 | .049 | .322 | 6.636 | .000 |
| | Easy accessibility of content | .052 | .034 | .045 | 1.552 | .024 |

a. Dependent Variable: Consumption Habit

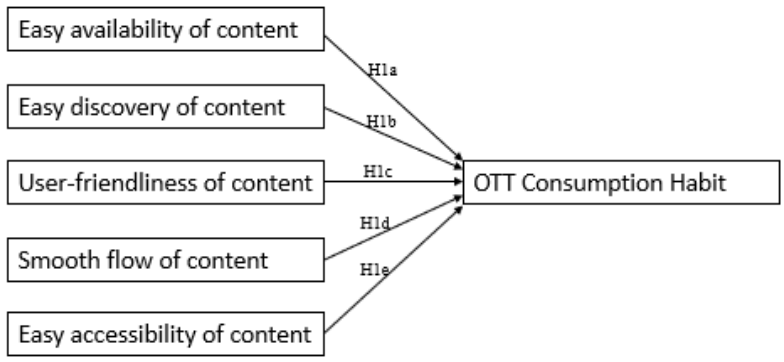


**Fig.1.Initial model**

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**RESEARCH ARTICLE**

# Holistic Therapy – An Integrative Approach to Physical and Mental Wellbeing

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**ABSTRACT**

Holistic therapy has gained prominence as a complementary approach to conventional medical treatments. It emphasizes the interconnection between the body, mind, and spirit, aiming to achieve overall wellness. This article delves into the effectiveness of holistic therapy, drawing on current research and critical perspectives to evaluate its role in promoting health and well-being. Holistic therapy, an integrative approach to physical and mental well-being, encompasses a range of practices that address the individuals as a whole, rather than focusing on isolated symptoms. This research article explores the efficacy of holistic therapy in improving physical and mental health outcomes, examining various modalities including acupuncture, yoga, meditation, and naturopathy. Critiques from contemporary researchers and practitioners are analysed to provide a balanced perspective on the benefits and limitations of holistic therapy. This research article depicts the impact and results of holistic therapies on physical and mental well-being, critically analysing the methodologies and outcomes of various holistic practices.

**Keywords:** Holistic Therapy, Integrative Approach, Physical-Mental Wellbeing, Methodologies, Holistic Practices, Outcomes

## INTRODUCTION

Holistic therapy is an integrative approach that seeks to address not only physical ailments but also emotional, mental, and spiritual health. Unlike conventional medicine, which often focuses on treating symptoms, holistic therapy aims to identify and treat the root causes of health issues. This approach views the person as a whole rather than treating specific symptoms or illnesses in isolation. Holistic therapies include a range of practices such as acupuncture, yoga, meditation, chiropractic care, naturopathy, and traditional Chinese medicine (TCM). The holistic approach aims to promote overall well-being and prevent disease by fostering balance and harmony within the body and mind (Dossey& Keegan, 2016).

Holistic therapy is based on the principle that health is not merely the absence of disease but a state of complete physical, mental, and social well-being. The World Health Organization (WHO) underscores this definition, emphasizing the need for a more inclusive approach to health **(WHO, 2020).** This philosophy aligns with the core tenets of holistic therapy, which advocates for treating the whole person rather than isolating and addressing specific symptoms.

Holistic therapy is not a new concept; its roots can be traced back to ancient healing traditions that emphasized balance and harmony within the body. Traditional Chinese Medicine (TCM), Ayurveda, and Indigenous healing practices all share the foundational belief that health is a state of equilibrium. The resurgence of interest in these practices in the modern era reflects a broader shift towards integrative health models. According to Brown (2020), "The re-emergence of holistic approaches in contemporary medicine signifies a paradigm shift from symptom-focused treatments to more comprehensive wellness strategies." (p. 45). One of the core principles of holistic therapy is the recognition of the mind-body connection. This concept is supported by a growing body of scientific evidence indicating that mental and emotional states can significantly impact physical health. For instance, stress has been linked to a variety of health problems, including heart disease, diabetes, and weakened immune function. In her research, Smith (2018) asserts, "The integration of psychological and physiological care is crucial for addressing the underlying causes of many chronic illnesses" (p. 63). This perspective is echoed by Miller (2019), who notes that "holistic therapies often succeed where conventional medicine falls short, particularly in managing chronic conditions and improving quality of life" (p. 122).

Holistic therapy represents a promising and comprehensive approach to health and wellness that addresses the interconnectedness of the mind, body, and spirit. By incorporating diverse practices such as mindfulness, yoga, nutrition, and lifestyle interventions, holistic therapy offers a multifaceted strategy for enhancing physical and mental well-being.

## LITERATURE REVIEW

The integration of holistic therapies into conventional medicine is a growing trend, reflecting a shift towards more patient-centred care. Health care providers are increasingly recognizing the value of addressing the physical, emotional, and spiritual needs of patients. According to a survey by the American Hospital Association (2021), "Approximately 42% of hospitals in the United States now offer some form of complementary and alternative medicine." (p. 67). This trend underscores the potential for holistic therapy to complement traditional medical treatments, providing a more comprehensive approach to health care. Nevertheless, the successful integration of holistic therapies requires collaboration between conventional and alternative health care providers. As emphasized by Taylor and Clark (2018), "Effective communication and mutual respect between practitioners of different medical traditions are essential for delivering integrated care that benefits patients" (p. 120). Bridging the gap between these diverse approaches can enhance patient outcomes and foster a more inclusive health care system. Research indicates that holistic approaches can significantly impact physical health. According to Fritts (2018), incorporating holistic practices like yoga and acupuncture alongside conventional treatments has been shown to enhance recovery rates and overall health outcomes. Fritts states, "The synergy of combining these modalities can lead to improved patient satisfaction and a more comprehensive healing experience" (p. 45).

There is a growing recognition of the potential benefits of integrating holistic therapies with conventional medical treatments. This integrative approach can provide a more comprehensive care plan that addresses all aspects of a patient's health. For example, cancer patients often benefit from combining conventional treatments with complementary therapies such as acupuncture, massage, and meditation to manage symptoms and improve quality of life (Cassileth& Deng, 2004). This integrated model of care is increasingly being adopted in healthcare settings to enhance patient outcomes and satisfaction (Maizes et al., 2009).

A study by Smith et al. (2020) found that patients engaging in holistic mental health practices experienced a 30% reduction in anxiety symptoms compared to those receiving standard care alone. The authors note, "The integration of holistic methods offers a substantial benefit in reducing mental health symptoms and improving quality of life" (p. 72). Meditation and mindfulness practices, for instance, have been shown to reduce symptoms of anxiety and depression by promoting relaxation and stress reduction (Goyal et al., 2014). Cognitive-behavioral therapy (CBT) integrated with holistic practices can help individuals develop healthier thought patterns and coping mechanisms. Additionally, therapies such as aromatherapy and massage therapy can improve mood and emotional well-being by inducing relaxation and decreasing cortisol levels (Cooke & Ernst, 2000).

This philosophy of care emphasizes the connection between these aspects of a person's health, advocating for a comprehensive approach that combines conventional medical treatments with alternative and complementary therapies. The aim is to achieve not only the alleviation of symptoms, but also the promotion of overall well-being. Holistic therapies have been found to have significant benefits for physical health. Studies have shown that practices such as acupuncture can alleviate chronic pain, reduce inflammation, and improve overall physical functioning (Vickers et al., 2018). Yoga and tai chi are particularly noted for their ability to enhance flexibility, strength, and cardiovascular health (Cramer et al., 2017). Additionally, holistic dietary approaches, such as those advocated in naturopathy, emphasize the consumption of whole, unprocessed foods, which can lead to improved digestion, weight management, and reduced risk of chronic diseases (Hawk et al., 2012). Nutrition and lifestyle are also critical components of holistic therapy. A balanced diet, rich in whole foods and nutrients, supports the body's natural healing processes and contributes to overall health. Research by Patel (2020) indicates that "dietary choices have a profound impact on both physical and mental health, highlighting the need for a holistic approach to nutrition" (p. 77). Additionally, lifestyle modifications, such as regular physical activity, adequate sleep, and stress management techniques, are integral to maintaining health and preventing disease. As Roberts (2019) observes, "Holistic therapy encourages individuals to adopt healthy habits that support their long-term well-being" (p. 101).

Holistic therapy also fosters a sense of empowerment and self-awareness among patients, encouraging active participation in their healing processes. According to Teixeira (2019), patients engaging in holistic practices often report improved self-esteem and a greater sense of control over their health. This holistic approach aligns with the biopsychosocial model, which considers the complex interplay between biological, psychological, and social factors in health and illness (Engel, 1977). Holistic therapy's integrative nature is one of its most praised aspects. By combining different therapeutic approaches, practitioners aim to treat the root causes of health issues rather than just the symptoms (Weil, 2014). Weil emphasizes, "A truly holistic approach can lead to profound healing by addressing all aspects of a person's life, including lifestyle, diet, and emotional health" (p. 23).

## RESULTS AND IMPACT

While holistic therapy is praised for its comprehensive approach, it faces criticism for the lack of standardized protocols and regulatory oversight. Miller (2018) argues that the absence of standardized training and certification for holistic practitioners can result in inconsistent care quality. Miller states, "Without standardized protocols, patients may receive varied and potentially ineffective treatments depending on the practitioner's training and experience" (p. 94). Furthermore, financial barriers can limit access to holistic therapies. Insurance coverage for these treatments is often insufficient, making them inaccessible to many patients (Johnson, 2017). Johnson points out, "The high cost of holistic therapies can prevent lower-income individuals from benefiting, thereby exacerbating health disparities" (p. 90). Accessibility and affordability are significant barriers to holistic therapy. Many holistic treatments are not covered by insurance, making them financially inaccessible to a broad segment of the population. As Singh (2019) points out, "the high cost of holistic therapies, coupled with the lack of insurance coverage, limits access for many individuals, particularly those from lower socioeconomic backgrounds" (p. 78). This economic barrier exacerbates health disparities and prevents many from benefiting from integrative health approaches.

Despite the benefits, integrating holistic and conventional treatments presents challenges. Martin (2016) points out that coordinating care between holistic practitioners and traditional medical professionals can be difficult due to differing philosophies and treatment goals. Martin observes, "Achieving seamless integration requires effective communication and a willingness to bridge the gap between conventional and alternative medicine" (p. 56). Roberts (2022) argues that the lack of rigorous scientific evidence and standardized protocols in holistic practices poses challenges to their widespread acceptance. Additionally, Green (2021) highlights the potential risks of relying solely on holistic treatments without conventional medical oversight, which can lead to delayed diagnoses and treatment of serious conditions. A lack of standardization and regulation in holistic therapy may contribute to inconsistent outcomes and experiences for patients (Garcia et al., 2022). Without clear guidelines and oversight, individuals may struggle to find qualified practitioners who can provide safe and effective holistic care. It's important to recognize that holistic therapy is not a panacea for all health issues. While it can complement conventional treatments and promote overall well-being, it may not be sufficient for managing complex medical conditions that require specialized interventions. While holistic therapies show promise in promoting well-being, more rigorous research is needed to establish their efficacy and safety."(Jones et al., 2020). Each individual responds differently to holistic therapies, making it challenging to predict outcomes accurately. What works well for one person may not have the same effect on another, highlighting the need for personalized treatment plans.Dr. John Smith (2021), a holistic therapist, emphasizes, "The key challenge we face is educating both patients and healthcare professionals about the benefits and limitations of holistic therapy."

## DISCUSSION

Holistic therapy promotes patient empowerment and involvement in their own health. Patients are encouraged to take an active role in their wellness journey, fostering a sense of agency and responsibility. According to Dillard (2020), "Holistic therapy empowers patients by involving them in their treatment plans and encouraging self-care practices, which can enhance their overall well-being". (p.93). This patient-centred approach contrasts with the often passive role patients play in conventional medical settings. One of the main challenges facing holistic therapy is the need for more rigorous scientific research to validate its efficacy. While many studies have shown promising results, further research is necessary to establish standardized protocols and evidence-based guidelines for holistic practices. Randomized controlled trials (RCTs) and systematic reviews are essential to demonstrate the effectiveness of holistic therapies and integrate them into mainstream healthcare with confidence (National Center for Complementary and Integrative Health, 2021). Ensuring the quality and safety of holistic therapy requires standardized training and regulation of practitioners. This involves establishing certification and accreditation processes that ensure practitioners have the necessary skills and knowledge to provide effective and safe treatments. Regulatory bodies should develop clear guidelines and standards for holistic therapies, similar to those in place for conventional medical practices (Lindquist et al., 2005).

To address these challenges, further research is needed to establish the efficacy and safety of holistic therapies. Increased collaboration between holistic practitioners and conventional medical professionals can also enhance integrative care models (Smith et al., 2020). Encouragingly, recent policy changes are beginning to recognize the value of holistic approaches, leading to better funding and research opportunities (Fritts, 2018). One of the primary challenges in holistic therapy is the integration of different modalities. Dr. Wayne Jonas (2017), a prominent advocate of integrative medicine, emphasizes this point: "Integrative medicine is not just a compilation of conventional and alternative therapies; it is an integration of the best practices and evidence-based approaches." This highlights the need for a thoughtful and evidence-based synthesis of various therapeutic techniques. Moreover, holistic therapy emphasizes preventive care and lifestyle interventions. Dr. Dean Ornish (2008) known for his work in reversing heart disease through lifestyle changes, emphasizes the importance of holistic approaches: "Changing lifestyle can be a more powerful intervention than any drug or surgery." This perspective underscores the potential for long-term health benefits through holistic interventions.

Holistic therapy aligns with the principles of patient-centred care, which emphasize respect for patients' values, preferences, and needs. By involving patients in their treatment plans and considering their holistic needs, healthcare providers can foster a more collaborative and supportive relationship. This approach can improve patient satisfaction, adherence to treatment, and overall health outcomes **(Epstein & Street, 2011).** Increasing scientific research on holistic therapies is vital to validate their efficacy and gain acceptance within the medical community. This involves conducting rigorous clinical trials and studies to generate evidence-based data. **Smith (2021)** asserts that "investing in scientific research on holistic therapies can bridge the gap between holistic and conventional medicine, fostering greater acceptance and integration" (p. 141). The integration of holistic therapy into mainstream healthcare has the potential to enhance patient outcomes by addressing the multifaceted nature of health. However, it is crucial to balance holistic practices with evidence-based medical treatments. As highlighted by critics, the need for more rigorous research and standardized protocols is essential for the broader acceptance of holistic therapies.

## CONCLUSION

The future of holistic therapy lies in its integration with conventional medicine to create a comprehensive, patient-centred approach to healthcare. This will require continued research, collaboration between conventional and holistic practitioners, and the development of integrative healthcare models. By combining the strengths of both approaches, healthcare providers can offer more effective and holistic care that promotes long-term health and well-being.

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**RESEARCH ARTICLE**

# Comprehensive Analysis of Microstrip Patch Antenna for Wireless Application

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**ABSTRACT**

This paper provides a comprehensive examination of recent development in microstrip patch antenna (MPA) design, tailored to diverse wireless applications. This survey paper presents a range of microstrip patch antennas that incorporate materials, feeding techniques, gain enhancement techniques, BW enhancement techniques, Performance enhancement. Further, the geometries of MPA are evaluated and scrutinized for their design characteristics, including size reduction, beam scanning capability, simplified feed structure, integration of filtering and radiation properties, multiple-input multiple-output (MIMO) functionality, and minimizing mutual coupling.

**Keywords :** Microstrip patch antenna, RFID, WBAN, ISM, Feeding Technique.

## INTRODUCTION

An antenna plays a crucial role in all electronic communication systems. Various types of antennas are developed according to specific application needs. Today's wireless communication demands antennas capable of operating across multiple frequency bands while maintaining a compact size. Microstrip patch antennas (MPAs) are favored in wireless communication systems due to their small size & low-profile, ease of fabrication, and compatibility with integrated circuit technologies. These antennas are constructed with a metal patch affixed onto a dielectric substrate, typically supported by a ground plane. The simplicity of their structure and the ability to integrate them with various wireless devices make MPAs highly versatile for a wide range of wireless applications [1,25, 8].

One of the key advantages of microstrip patch antennas is their adaptability to different frequency bands and applications through variations in their geometrical parameters and substrate materials. They can be configured to function across different frequency bands, including the ISM band, Wi-Fi bands, cellular bands, and beyond. Additionally, advancements in materials and innovative feeding techniques have further enhanced the performance and functionality of MPAs in modern wireless systems [24, 4, 7, 8].

This paper endeavors to explore a variety of microstrip patch antennas, categorized by the materials utilized, feeding techniques, performance enhancement techniques as documented across multiple academic journals and conference papers. Specifically focusing on material-inspired designs, the study systematically reviews and contrasts these antennas, assessing their performance metrics including gain, directivity, bandwidth, and physical dimensions [1, 5,13].

Due to its two-dimensional structure, microstrip patch antennas are cost-effective and straightforward to produce. They offer numerous benefits, including lightweight construction, the capability of direct printing onto circuit boards, and a low-profile design. This study examines and analyzes several MPA antennas documented in existing literature, deemed appropriate for diverse wireless applications. Section 2 outlines the fundamental configuration of the MPA antenna, discussing different available feeding techniques and their respective performances. Recent progressions in MPA antenna development are reviewed in Section 3. Concluding remarks, including a summary of findings are presented in Section 4.

**Fundamental of microstrip patch antenna, Feeding Network and literature review**

Microstrip antenna research has seen significant innovation and activity in recent years within the communication field. To analyze and validate its design, various geometric shapes such as rectangles, squares, triangles, circles, ellipses, and other common forms are employed. Rectangular and circular patches are particularly prevalent in these studies. The effectiveness of the microstrip patch antenna is contingent based on its dimensional characteristics. The basic configuration of the antenna is depicted in Figure 1. The MPA dimensions encompass the patch length (L), patch width (W), and substrate height (h) [24, 25].

**Feeding Network**

Several techniques exist for feeding microstrip patch antennas, divided into two primary categories: contacting and non-contacting. Contacting techniques entail the direct transmission of RF power to the radiating patch through connecting elements such as microstrip lines. Conversely, non-contacting approaches involve transferring power to the patch from the feed line through electromagnetic coupling. Among non-contacting methods, aperture and proximity coupled feeds are frequently employed.

**Microstrip Line Feed**

In this feed configuration, a conductive strip is attached directly to the microstrip patch's edge. The conducting strip's width is smaller than that of the patch. This feed arrangement offers the advantage of integrating the feed onto the identical substrate, facilitating a planar structure [10]. The patch with inset-cut serves the purpose of aligning the impedance of the feed line with the patch, eliminating the requirement for extra matching components [4, 8].

**Co-axial Feed**

The coaxial feed employs a non-planar feeding approach wherein a coaxial line is employed to feed a patch. The internal conductor of co-axial connector penetrates the dielectric, establishing a metallic connection with the patch, while the external conductor of cable is linked to ground plane. The co-axial probe makes direct connection with antenna and is placed at specific location where antenna exhibits an input impedance of fifty ohms [28, 30].

**Aperture Coupled Feed**

The aperture feed technique involves two dielectric substrates: the feed and the antenna dielectric substrate. The dielectric substrate incorporates a slot at its center, which is separated by a ground plane. Metallic patch is situated on upper surface of antenna substrate with ground plan on other side. It provides better bandwidth. The drawback of this feeding method is its requirement for multilayer fabrication [4, 18].

**Proximity Coupled Feed**

This technique is employed in microstrip patch antenna design in which the feeding mechanism involves electromagnetic coupling between feed line with patch. In this technique, patch and feed line are placed in close proximity without direct physical contact. This approach offers advantages such as simplified fabrication and reduced complexity compared to other feeding methods [7].

## LITERATURE REVIEW

Within this segment, antenna with diverse designs is examined. The categorization methods for MPA operation, accompanied by particular illustrations, is shown in Figure 2. Shan Gao [26] presented precise coverage challenge arising from Wi-Fi 6 asymmetrical bands through manipulating TM 0.5, 0 along with TM 0.5, 1 modes of the half mode patch antenna, keeping its volume significantly small. The design guideline consists of three sequential steps. Initially, the appropriate patch size is chosen to ensure resonance of initial modes at 2.45 and 5.50 GHz, employing 2-ports. Subsequently, feeding locations are fine-tuned to minimize the sizes of impedance trajectories on the Smith Chart. Finally, lumped components are employed to ensure both resonances exhibit favorable matching conditions. The suggested antenna features dual-port functionality and aperture sharing characteristics, aligning well with the optimal RF front-end architecture for Wi-Fi 6.

Amit Baran Dey [33] antenna designed for IoT and medical applications is compact, wide-band and low-profile, constructed from elastomeric fabric. The synthesis of a flexible antenna integrated with artificial magnetic conductor (AMC) is achieved by utilizing textile layers. Operating with range of frequency 4.76–6.08 GHz, antenna covers both the ISM band -5.8 GHz and Wi-Fi 5 GHz band, catering to IoT applications. Proposed design offers 24.4% of an impedance bandwidth, accompanied by 10.59 dBi of high gain. Its small dimensions are $1.44\lambda_0 \times 0.46\lambda_0 \times 0.0512\lambda_0$.

Haiyan Li [19] A proposed antenna for WBAN applications is low-profile, compact and completely reliant on textile materials. It operates across multiple bands, including the 2.45/5.8 GHz ISM bands, mobile Wi MAX 3.3 – 3.4 GHz, and 3.85 – 4.0 GHz 5G sub-6 NR frequency band. A C-shaped slot and an elliptical slot are utilized to adjust 3- modes to intended operating frequencies. The suggested antenna is constructed using a single layer of denim integrated with a conductive fabric layer, resulting in an exceptionally low-profile design ideal for wearable applications. The recorded peak realized gains and bandwidths (four frequencies) are as follows: -0.81 dBi, -2.81 dBi, -1.16 dBi, and 2.83 dBi, with bandwidths of 90 MHz, 190 MHz, 230 MHz, and 570 MHz, respectively. Test outcomes reveal that minimal changes occur in the reflection coefficient whenever antenna is positioned on a model simulating human arm and bent around it. Sen Yan [22] presented a textile-based PIFA (planar inverted-F antenna). Through simultaneous design of PIFA antenna incorporating ground plane, broad matching BW of approximately 433 MHz is attained for compact electrical size of $0.202\ \lambda_0 \times 0.115\ \lambda_0 \times 0.01\ \lambda_0$. Incorporating slot in PIFA facilitates operation within a 2400 MHz. Measured lower & upper bands bandwidths are 35 and 309 MHz, respectively and associated radiation efficiencies are 48% and 64%.

Chun-Cheng Lin [4] demonstrated unique feeding technique for a DRA through incorporating microstrip off-centered feedline. It is designed with slot-coupled circularly polarized for applications within the 2400 MHz ISM band. The suggested antenna exhibits an 10-dB impedance BW of 141 MHz and an axial ratio (3-dB) of 85 MHz and also a satisfactory RHCP of antenna. Amit Birwal [32] presented a CPW- fed bi- direction CP antenna as RFID reader. A square antenna with overall size of 60mm × 60mm with 1.5mm thickness. The measured outcome settled an

impedance BW (2.1 -3.2GHz) and ARBW(2-2.7GHz), with 3 dBi gain at 2500 MHz. Proposed antenna achieves peak gain of 3.17 dBi in both the positive and negative z-directions. The path loss of antennas is observed to rise within the desired band due to the presence of interference.

Lin Guo [14] realized a dual-antenna setup consists of two symmetrical antenna elements & a ground plane featuring an embedded T-shaped slot. It is fabricated on a single-layer PCB with compact dimensions with 9 by 40 millimeters. The antenna is a loop antenna fed through coupling, featuring a compact size just 9 by 7 millimeters. A prototype demonstrates that the achieved 10-dB BW are 190 MHz and 1.82 GHz for 2.38–2.57 GHz, 4.34 – 6.16 GHz respectively. Additionally, the archived mutual couplings below 18 dB and 20 dB with 2.4 GHz and 5.2/5.8 GHz for WLAN. The obtained efficiencies and gains at 2.4, 5.2 and 5.8 GHz are better than 4.1dBi and 65% / 54%.

### Performance Enhancement of MPA

**MPA antennas with different feeding**

This section discusses the latest progress in MPA antenna. Investigators have undertaken as in many endeavors to deal with a variety of challenges, together with evolving dual [8, 18], Isolation [28], Wideband [10, 27], BW enhancement [4, 8, 10, 28], Circular polarization [4, 18, 27, 32] etc. Furthermore, nearly all things documented have tried to emphasizes the development of smaller and more condensed MPA alongside performance enhancements, which are summarized in table 1.

**Performance analysis of microstrip patch antenna**

This section addresses MPA antennas, which are aimed at enhancing antenna performance. These cover broadening bandwidth, increasing gain, improving efficiency, and mutual coupling of array, size reduction. The antennas associated with these contributions are outlined in Table 2

**Defective ground structure**

For enhancing the microstrip antenna's performance, the DGS method is utilized. DGS refers to a pattern constructed on ground plane of MPA, which is subsequently removed via etching. Implementing DGS alters the characteristics of the transmission line and disrupts the current flow, influencing antenna's current. It enables precise control over activation and EM travelling through the substrate layer. Utilizing DGS with a microstrip patch antenna addresses limitations of conventional designs by enhancing parameters like return VSWR, loss, impedance BW, and lowering the required copper region of the ground [6, 9, 24].

**Slot loading**

The slot loading technique involves etching a slot from the radiating patch, effectively lengthening the current distribution path. This leads to an expanded bandwidth and a smaller size to MPA. The fundamental resonant frequency is contingent upon the length and positioning of the slot. To enrich gain and BW of the patch antenna, slot loading technique is employed [10, 14, 16, 23].

**Meander line technique**

The meander line technique is primarily employed in compact antennas to reduce their size. This is accomplished by folding the conductor in a back-and-forth manner. While this technique offers advantages in terms of configuration and easy integration into electronic devices, it is hampered by low radiation efficiency. As the antenna size decreases, its radiation resistance diminishes [12, 15].

## CONCLUSION

Due to its lightweight nature, ease of fabrication, and affordability, the microstrip patch antenna holds significant promise for applications in today's wireless communication. However, it faces challenges like limited small BW, gain

and limited power handling abilities. This paper examines different methods aimed at addressing the constraints of traditional microstrip patch antennas. It's noted that the DGS technique enhances the gain, lower the size and return loss. The slot loading technique serves to reduce size and enables the antenna to resonate across different frequencies. Employing the meander line technique enables the creation of compact microstrip patch antennas.

This review has demonstrated that the rectangular MPA has captured the attention of researchers, sparking significant interest in research. The subsequent findings have emerged from a range of studies:

1. Modifying the patch shape improves bandwidth performance.
2. Defected ground structures contribute to shaping the radiation pattern.
3. Using slots and altering the patch design enhances the performance of the antenna.
4. Defected Ground Structures (DGS) play a critical function in shaping the performance of microstrip patch antennas. By introducing discontinuities in the ground plane, DGS can change electromagnetic properties of the antenna system. The effects of DGS include improved impedance matching, reduced mutual coupling, enhanced radiation pattern control, and increased bandwidth.
5. The greatest challenge in designing small, compact and low-profile MPA lies in enhancing performance parameters such as gain, frequency response, BW, isolation, directivity, return loss -(S11), VSWR, and efficiency.

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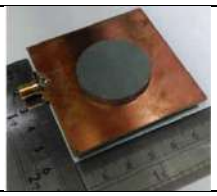
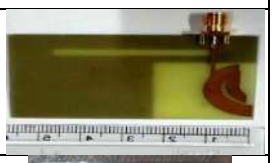
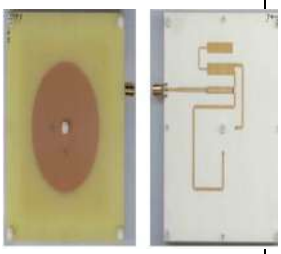
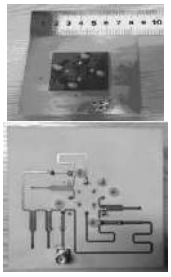
**Table-1. Performance evaluation of MPA antennas utilizing various feed setups.**

| Ref. | Feed type | Technique and material used | Reported results | Application | Specimen prototype |
|---|---|---|---|---|---|
| [4] | Microstrip feedline-Off-centered | cylindrical dielectric resonator with slot-coupled Substrate:FR4 | Gain: 2.7dBi Operating frequency: 2.4GHz Impedance bandwidth:141MHz | ISM Band |  |
| [7] | Proximity feedline | Feed length F1=12mm, F2=16mm, F3=23mm | Operating frequency: 2.34, 2.4, 2.48GHz Return loss: -37.16, -53.29, -24.40 dB | ISM Band | -- |
| [8] | Microstrip feedline | Defected ground plane Slot in ground plane FR4 | Gain: 1 to 4 dBi Operating frequency: 1.8 and 2.4GHz | Mobile/wireless, Lower UWB |  |
| [10] | Microstrip line | Borosilicate glass substrate Rectangular slot in Ground plane | Gain: -- Operating frequency: 1.8 to 10.8 GHz | -- | |
| [18] | Dual-feed network | Connectors (SMA) connected to ports 2 radiating patches-lower square & upper circular patch Directional dual band circularly polarized FR4 | Gain: 3.8 & 8.9 dBi Operating frequency: 0.925 & 2.45 GHz | RFID |  |
| [27] | Microstrip line | Shorting loads onto the patch, the resonance frequency with modes such as TM 20, TM 30, TM 40 & TM 50 is lowered, effectively merging with TM 10 mode. Balun us used to split | Gain: -- Operating frequency: 2.2 & 5.5 GHz BW: 85% | UWB |  |

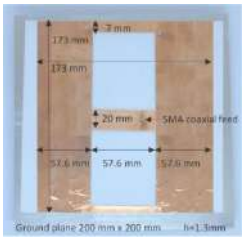
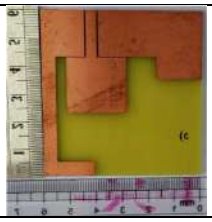
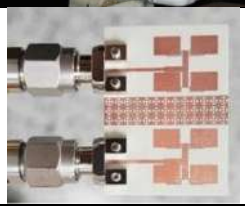
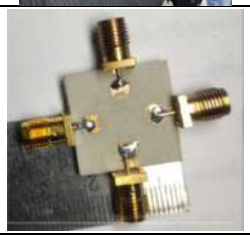
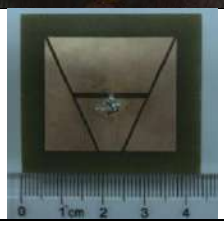
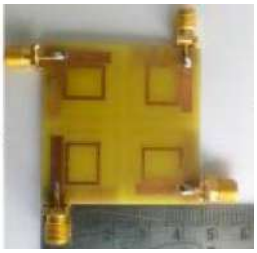
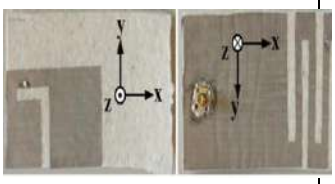
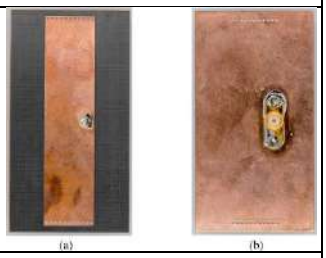
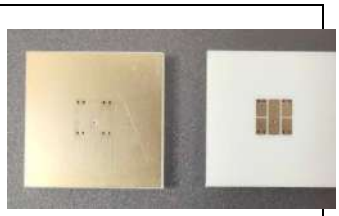
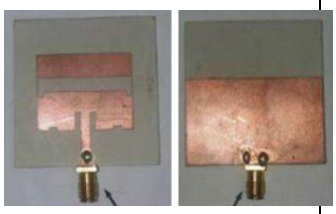
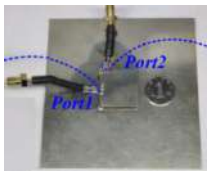
| | | | | | |
|---|---|---|---|---|---|
| | | signal into 0, 90, 180, 270 Degree. | | | |
| [28] | Microstrip & coaxial probe | To excite TE110 and TE120 modes, a circular ring slot is incorporated with frequency (5-6GHz). circular ring slot with SIW cavity to resonant antenna at 5.8GHZ. Substrate: single layered RT / Duroid | Gain:6.97 & 6.2 dBi Operating frequency: 5.2 & 5.8 GHz BW: 6 % & 3.4% | Wi-Fi/ISM | |
| [30] | Coaxial probe | Higher order mode is adjusted to reduce the impact of currents that are out of phase. Operates Antenna at modified $TM_{30}$ & $TM_{50}$ with directivity 15 and 18dB Substrate: foam | Operating frequency: 2.6 GHz BW: 15.2% & 3.6% Measured Directivity for $TM_{30}$ 14.6dB | line-of-sight |  |
| [32] | CPW Feed | Antenna with bi-directional CPW for circular polarization. RFID-Reader Polarization: Circular Substrate:FR4 | Gain: 3dBi at 2.5GHz Operating frequency: 2.1 to 3.2 GHz | RFID |  |

**Table 2. Performance Comparative analysis of MPA antennas**

| Ref. | Specifications & features of antenna geometry | Obtained Outcomes | Applications | Specimen |
|---|---|---|---|---|
| [1] | Dual band structure for BW enhancement<br><br>Series-fed patch array | Gain:13.3dB Operating frequency: 5.8 GHz Impedance bandwidth: 340 MHz | ISM Band | |
| [3] | Antenna-doppler radar designed for moving target sensing applications featuring high isolation. 2 × 2 array for Tx, Rx antenna. Rogers RO4350B | HPBW: H-plane -47.54°, E-plane -58.53° Operating frequency: 23.91 to 24.42 GHz Impedance bandwidth:510 MHz | ISM Band |  |

| | | | | |
|---|---|---|---|---|
| [5] | Triple band with CP<br>Rogers RO3010<br>Antenna<br>Size:10mm×10mm×1.016 mm. | Gain: -31.82dBi, -21.8dBi, -18.5dBi<br>Operating frequency: 860MHz, 1.85GHz, 2.45GHz<br>Impedance bandwidth: 15.6%, 7.5%, 18.7% | Bio-Telemetry | |
| [6] | MIMO<br>Rogers Duroid 5880<br>Antenna Size:20×20×0.8mm3. | Gain:9.24 dB<br>Operating frequency: 28GHz<br>Impedance bandwidth: 2.1GHz<br>Return Loss: -35dB<br>Isolation: >18dB | 5G |  |
| [9] | Slotted Patch with (PBG) photonic band gap substrate<br>Antenna Size:35×45×3mm3. | Gain:3.714 dB<br>Operating frequency: 2.49GHz<br>Impedance bandwidth: 174.3MHz<br>Return Loss: -20.11dB<br>VSWR:1.22 | ISM band | -- |
| [10] | Borosilicate glass substrate<br>Rectangular slot in Ground plane<br>Antenna Size: 46 mm × 50 mm | Gain: --<br>Operating frequency: 1.8 to 10.8 GHz<br>Impedance bandwidth: -<br>Return Loss: -<br>VSWR:<2 | -- | |
| [11] | Multiple parasitic patches<br>Shorting vias (bandwidth enhancement).<br>FR4 | Gain: --<br>Operating frequency: 5.56 to 6.55 GHz<br>Impedance bandwidth: 17.4%<br>Return Loss: <-10dB | -- |  |
| [12] | A slotted rectangular box linked with a reverse S-shaped meandering line<br>Antenna Size: 40× 10× 1.6 mm3<br>FR4 | Gain: 1.347dB<br>Operating frequency: 2.4 GHz<br>Impedance bandwidth: 146MHz<br>Return Loss: <-10dB | IoT | |
| [13] | A polarization diversity technique is employed in the MIMO antenna<br>Antenna Size: 70× 70 mm2<br>FR4 | Gain: 3.98, 4.13dBi<br>Operating frequency: 2.54, 5.26GHz | WLAN, WiMAX | |
| [14] | T-shaped slot<br>Dual antenna | Gain: 4.1dBi<br>Operating frequency: 2.38- | WLAN | -- |

| | | | | |
|---|---|---|---|---|
| | loop antenna comprising a driven segment and an accompanying parasitic loop. Antenna Size: 9× 40 mm2 FR4 | 2.57 GHz, 4.34-6.16GHz Impedance bandwidth: 190MHz, 1.82GHz | | |
| [15] | Patch antenna design for gain-enhanced Microstrip structure with periodically spaced rampart | Gain: 10dBi Operating frequency: 5.8GHz Impedance bandwidth: 4% | -- | |
| [16] | Dual band antenna for WBAN-open-ended slot for second resonant Rogers Duroid 5870 | Gain: 2.13 and 5.16dBi Operating frequency: 2.44 and 5.787 GHz Impedance bandwidth: 30MHz and 114MHz Efficiency: 60% and 76% | WBAN | |
| [20] | MIMO- printed monopole antenna, which is partially grounded and incorporates a split ring resonator Antenna Size :0.33λ x 0.33λ x 0.01λ. FR4 | Average Gain: 2.75dBi Peak Gain: 4dBi @3.3GHz Operating frequency: 2.2 to 6.28GHz Impedance bandwidth: 96.2% Isolation Between Element :>14dB | WiMAX, WLAN, Bluetooth and ISM |  |
| [22] | Planar inverted-F textile antenna. Slots are incorporated in ground to enhance BW. substrate: 6-mm-thick felt Slot introduced to resonate at 2.4 GHz | Gain: -0.5 & 6.1dBi Operating frequency: 430MHZ & 2.428GHz Impedance bandwidth:35 & 309 MHz | ISM |  |
| [29] | Shorted both sides of patch Two modes TM03 and TM11 are excited and combined to enhance bandwidth Antenna Size: 1.29λ0 × 0.73λ0 × 0.036λ0 Coaxial probe feeding | Gain: 8 to 9.7dBi Operating frequency: 5.17 to 5.9 GHz Impedance bandwidth: 13.2% cross-polarization level: -25dB | -- |  |
| [31] | Multilayer-Electromagnetically Coupled antenna Substrate: FR4 and felt textile | Gain: 4.28 and 7.33dBi Operating frequency: 2.45GHz Impedance bandwidth: 4%, 5.12% radiation efficiencies: 63.4%, 71.8% | ISM | --- |

| | | | | |
|---|---|---|---|---|
| [17] | Single layer miniaturizes coupled microstrip patches antenna.<br>Antenna Size: 0.146×0.146×0.04 λ0<br>3<br>Substrate: Rogers4330 | Gain: 5.4dBi<br>Operating frequency: 8.45GHz<br>Impedance bandwidth: 186 MHz<br>HPBW:107° for E-plane and 87° for H-plane. | -- |  |
| [21] | Microstrip patch array antenna arranged in a 3 × 3 configuration with parasitic coupling.<br>Patches: Probe feeding with center patch, functions as the active element and others as parasitic elements.<br>Substrate: Rogers- Duroid 5880 | Gain: 14.8dBi (Maximum)<br>Operating frequency: 18 to 21GHz<br>Impedance bandwidth: 15.4%<br>Aperture efficiency: 53% | Radar, Satellite | |
| [19] | Textile circular patch multiband antenna.<br>Multi-bands are produced by activating TM 11, TM 21, and TM 31 modes through the elliptical and C-shaped slots.<br>Antenna size: 0.64 λg × 0.64 λg × 0.0125 λg ( 2.45GHz)<br>Substrate: Denim | Gain: -0.81, -2.81, -1.16 and 2.83dBi<br>Operating frequency: 2.45, 3.32, 3.93 and 5.8 GHz<br>Impedance bandwidth: 90, 190, 230 and 570 MHz | WBAN | |
| [23] | Wearable compact antenna with dual-band<br>Using an inverted U-shaped slot to produce frequencies in two bands<br>Substrate: Rogers Duroid RO3003 -semi-flexible<br>Antenna size: 41×44mm² | Gain: 3.74 and 5.13dBi<br>Operating frequency: 2.4 and 5.8 GHz<br>Impedance bandwidth: 3.8%, 5.2%<br>Efficiency: 91.4%, 92.3% | WBAN-wireless body-area networks |  |
| [26] | Wi-Fi-6 band Dual port antenna.<br>Exactly Covering -Wi-Fi 6 bands<br>Antenna Size:<br>22.55 × 23.95 mm²<br>Substrate: FR4 | Gain: 4.4 and 8.35dBi<br>Operating frequency: 2.40 to 2.50 GHz and 5.15 to 5.85 GHz | WLAN, Wi-Fi |  |
| [33] | Elastomeric wide-band textile antenna<br>The radiation efficiency increased with AMC (Artificial magnetic conductor) - from 56% to 85%<br>Antenna size: 1.44λ0× 0.46λ0× 0.0512λ0<br>Substrate: Textile Jeans | Gain: 10.59dBi<br>Operating frequency: 4.76 to 6.08 GHz<br>Impedance bandwidth: 24.4%<br>Radiation efficiency: 85% | IoT | |

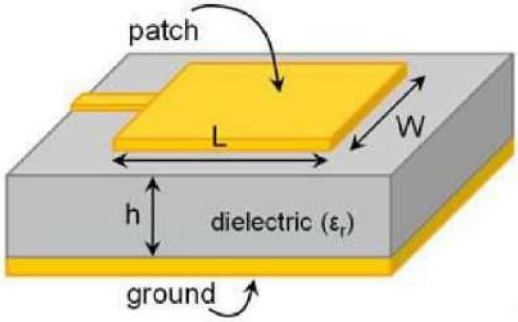
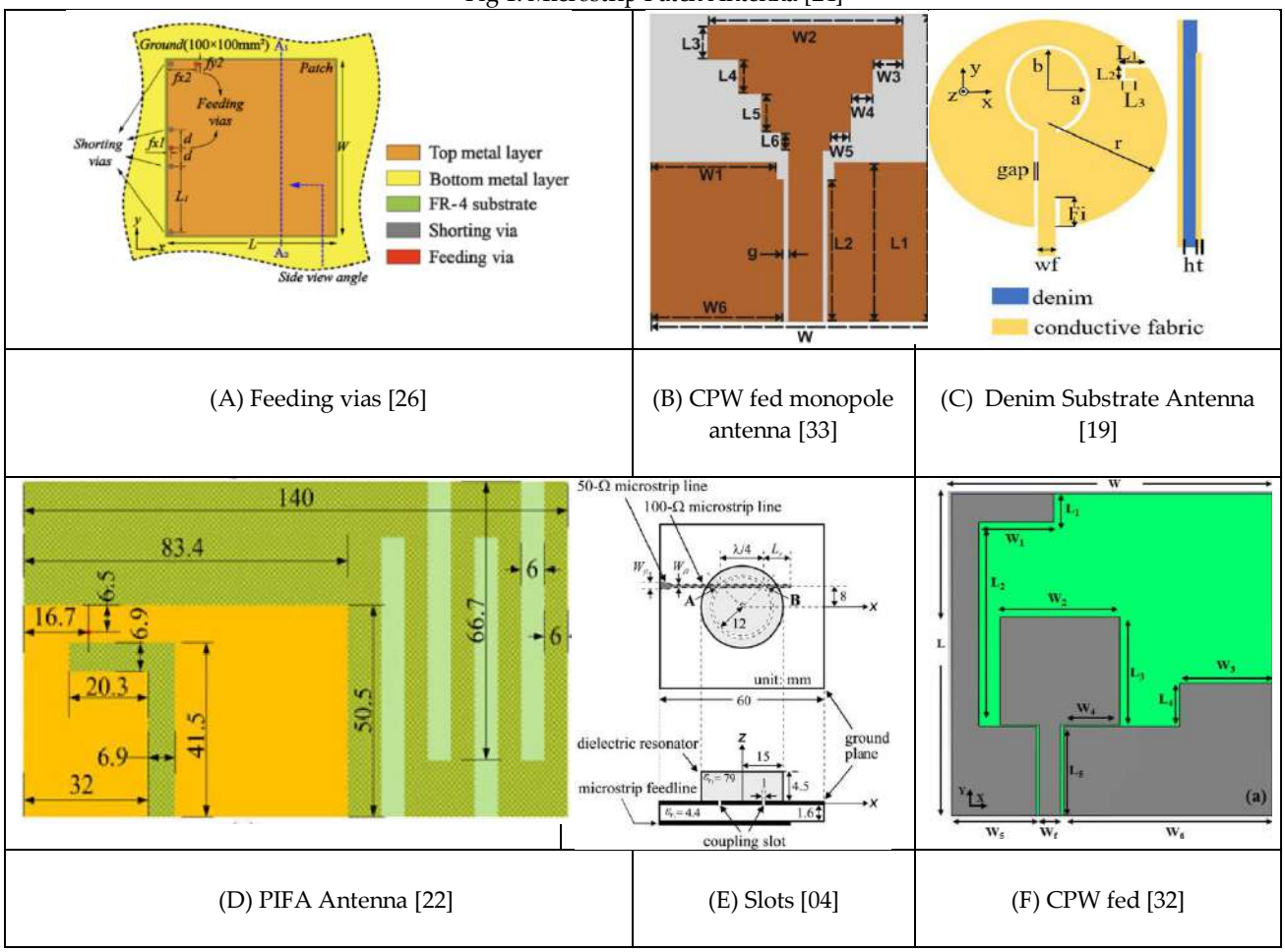



Fig 1. Microstrip Patch Antenna [24]




| (A) Feeding vias [26] | (B) CPW fed monopole antenna [33] | (C) Denim Substrate Antenna [19] |
|---|---|---|
| (D) PIFA Antenna [22] | (E) Slots [04] | (F) CPW fed [32] |

**Figure 2. Examples of MPA Antenna with different design: (A) Feeding vias (B) CPW fed monopole antenna (C) Denim Substrate Antenna (D) PIFA Antenna (E) Slots (F) CPW fed**

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**RESEARCH ARTICLE**

# Effective Completion of TNPSC Examination using Fuzzy Matrix

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**ABSTRACT**

This paper gives a brief survey on the TNPSC examinations Participate candidates. The method of application of Combined Effective Time Dependent Data (CETD) Matrix, Average Time Dependent Data (ATD) Matrix, and Refined Time Dependent Data (RTD) Matrix which are fuzzy models are studied using fuzzy matrices. The effects and objectives of data's using the concept of mean and Standard deviation

**Keywords:** Fuzzy matrix, ATD matrix, RTD matrix, CETD matrix.

## INTRODUCTION

In recent years fuzzy set theory and fuzzy logic are very much suitable and applicable for decision making problem. Decision making is an act to choose the correct option between two or more alternatives. There are many techniques used to improve decision making process, Fuzzy plays a vital role in solving decision making problem in different complicated aspect. L.A Zadeh (1965) introduced fuzzy set in the year 1965. Fuzziness can be showed in many ways. Membership function is one of the most useful representation in fuzzy set theory. It depends upon the nature and shape of the membership function. A fuzzy number is thus a special case of normalized fuzzy set of the real line and convex. Fuzzy numbers is also an extension of real numbers. It is a generalization of regular, real number. It does not refer to one single value but rather to a connected set of possible values. Each element has its own weight between 0 to 1 and this weight is called the membership function. Fuzzy matrix plays a vital role in scientific development. Application of Fuzzy Matrix Theory in COVID-19 Pandemic was developed by Anjan Mukherjee and Abhik Mukherjee [1]. Application of Fuzzy Matrices in Medical Diagnosis was defined by Beaula, T and Mallika [2].

"Operations on fuzzy numbers with function principle" was discussed by Chen, S.H(1985) [3]. "Two new operators on fuzzy matrices "and "Triangular Fuzzy Matrices" written byShyamal, A.KMadhumangal Pal [13] [12]. Application Of Fuzzy Membership Matrix In Medical Diagnoisis and decision Making was developed byElizabeth, S and Sujatha, L [4]. Fuzzy Numbers, Positive and Nonnegative was defined by Hadi Nasseri[5]. Study of Traffic Flow Using CETD Matrix was discussed byKuppuswami, G and Sujatha, Rand Vasantha Kandasamy, W.B[6]. "Basic concepts of fuzzy matrix" written by Priya [7]. Risk Factor of Breast Cancer Using CETD Matrix-An Analysiswas developed by Radhika, K and Anbalagan and Alexander and Suganthi Mariyappan[8]. Fuzzy Matrix Theory and its Application for Recognizing the Qualities of Effective Teacherwritten by Raich,Vivek .V and Archana Gawande and Rakesh Kumar Triapath [9]. An Application of Improved Method of Fuzzy Matrix Composition in Medical Diagnosis was discussed bySangodapo, O Taiwo and Yuming Feng[10].

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**Preliminaries**

**Definition 2.1**

If $\hat{X}$ is a collection of objects generically denoted by x, then a fuzzy set $\hat{A}$ in $\hat{X}$is a set of ordered pairs $\hat{X} = \{(\hat{x}, \mu_{\hat{A}}(x)) : x \in \hat{X}\}$ Where $\boldsymbol{\mu_{\hat{A}}(x)}$is the membership function which maps $\hat{X}$to a real number in the interval $[0, 1]$.

$$\mu_{\hat{A}}(x) : \hat{X} \to [0, 1]$$

**Definition 2.2**

An $m \times n$ matrix$\hat{A} = (a_{ij})$, $1 \le i \le m, 1 \le j \le n$ is said to be a fuzzy matrix if where $a_{ij} \in [0,1]$.First input the raw data. This data gives the matrix representation.

Convert the raw data matrix into average time dependent matrix by dividing each entry of the raw data matrix with the width of the respective class interval. Calculate the mean and standard deviation of every column in the ATD matrix by using the formula Mean = $\frac{\sum x}{n}$ and Standard deviation =$\sqrt{\frac{\sum x^2}{n} - \left(\frac{\sum x}{n}\right)^2}$. Convert the ATD (Average Time Dependent) Matrix into (Referred Time Dependent) RTD Matrix. This is also termed as the entries are 1 0, &-1 by using the formula

$$\begin{cases} a_{ij} \le (\mu_j - \alpha * \sigma_j) \; then \; e_{ij} = -1 \; else \\ a_{ij} \in (\mu_j - \alpha * \sigma_j, \mu_j + \alpha * \sigma_j) \; then \; e_{ij} = 0 \; else \\ a_{ij} \ge (\mu_j + \alpha * \sigma_j) \; then \; e_{ij} = 1 \; else \end{cases}$$

Obtain the CETD Matrix (combined effective time dependent) data matrix and the corresponding row sum by adding all the ATD matrix.

**APPLICATION OF CETD MATRIX IN CRACKING THE TNPSC EXAMINATION**

**Introduction**

The Tamil Nadu Public Service Commission (TNPSC) exams are conducted by our state government. And it works under the guidance of UPSC. The commission be made up of 15 members with 1 chairman. And its conducted several types of exam with many number of posts, promotion, hike in salary etc., the exams are group I, group II,

group II A, group IV, group VII etc.This paper mainly focusses on the study of which age group of the people crack the TNPSC examination in their first attempt. To explain this concept, the effective completion of TNPSC CETD Matrix is used. And also suggest some ideas for their effective completion those who are trying to complete the examination.

**An illustrative Example for the Algorithm discussed in Preliminaries**

To complete the TNPSC competitive examination the candidates facing many problems. Among them we categorized six most important attributes by interviewing and collecting the data from TNPSC candidates and coaching center mentor.

The following attributes

$S_1$ - Lack of study plan and strategy

$S_2$ - Gaps in their knowledge

$S_3$ - Exam Anxiety

$S_4$ - Lack of exam management skills

$S_5$ - How to approach exam question

$S_6$ - Short memory

**Table 1.** Initial Raw Data Matrix of Struggles of Competitive exam

| Age | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ |
|---|---|---|---|---|---|---|
| 18-22 | 23 | 9 | 15 | 13 | 19 | 16 |
| 23-27 | 17 | 12 | 12 | 18 | 20 | 14 |
| 28-32 | 11 | 14 | 10 | 21 | 15 | 9 |

*Step :* Convert the raw data matrix into average time dependent matrix by dividing 5 and the result shown in table 3.2

**Table 2 The ATD Matrix of table 3.1**

| Age | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ |
|---|---|---|---|---|---|---|
| 18-22 | 4.6 | 1.8 | 3 | 2.6 | 3.8 | 3.2 |
| 23-27 | 3.4 | 2.4 | 2.2 | 3.6 | 4 | 2.8 |
| 28-32 | 2.2 | 2.8 | 2 | 4.2 | 3 | 1.8 |

*Step :* Calculate the Mean and standard deviation of every column in the ATD Matrix by using formula

Mean $=\frac{\sum x}{n}$ and standard deviation $= \sqrt{\frac{\sum x^2}{n} - \left(\frac{\sum x}{n}\right)^2}$.

**Table 3** Mean and Standard deviation of ATD Matrix

| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ |
|---|---|---|---|---|---|---|
| Mean $\boldsymbol{\mu}$ | 3.4 | 2.33 | 2.40 | 3.5 | 3.6 | 2.6 |
| Standard Deviation $\boldsymbol{\sigma}$ | 1.200 | 0.50334 | 0.52915 | 0.80932 | 0.52915 | 0.7211 |

*Step :* Convert the ATD Matrix into RTD Matrix.

This matrix is also termed as fuzzy matrix as the entries are 1, 0 &-1 by using the formula

$$\begin{cases} a_{ij} \le (\mu_j - \alpha * \sigma_j) then e_{ij} = -1 else \\ a_{ij} \in (\mu_j - \alpha * \sigma_j, \mu_j + \alpha * \sigma_j) then e_{ij} = 0 else \\ a_{ij} \ge (\mu_j + \alpha * \sigma_j) then e_{ij} = 1 else \end{cases}$$

The RTD Matrix for $\boldsymbol{\alpha = 0.1, 0.15, 0.2, 0.25}$

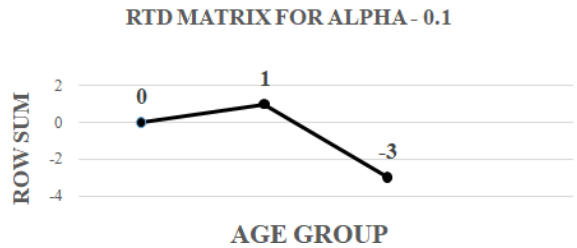
The RTD Matrix for $\boldsymbol{\alpha = 0.1}$ Row Sum Matrix

$$\begin{bmatrix} 1 & -1 & 1-1 & 1 & -1 \\ 0 & 0 & 0\ 0 & 1 & 0 \\ -1 & 1 & 0-1 & -1 & -1 \end{bmatrix} \qquad \begin{bmatrix} 0 \\ 1 \\ -3 \end{bmatrix}$$

| Age Group | 18-22 | 23-27 | 28-32 |
|---|---|---|---|
| Row Sum | 0 | 1 | -3 |

Table row sum of RTD Matrix for alpha = 0.1

The Graphical representation of the RTD Matrix at alpha = 0.1




The RTD Matrix for $\boldsymbol{\alpha = 0.1}$5 Row Sum Matrix

$$\begin{bmatrix} 1 & -1 & 1\ -1 & 0 & 1 \\ 0 & 0 & 0\ 0 & 1 & 0 \\ -1 & 1 & -1\ 1 & -1 & -1 \end{bmatrix} \qquad \begin{bmatrix} 1 \\ 1 \\ -2 \end{bmatrix}$$

**Table 5** Row sum of RTD Matrix for alpha = 0.15

| Age Group | 18-22 | 23-27 | 28-32 |
|---|---|---|---|
| Row Sum | 1 | 1 | -2 |

The Graphical representation of the RTD Matrix at alpha = 0.15

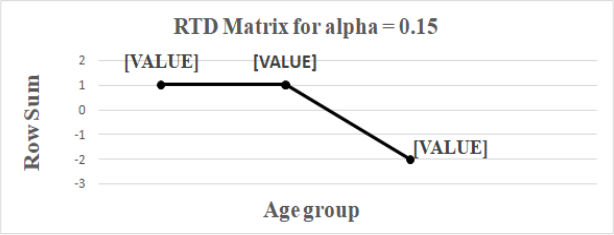



**Figure 3.2**

The RTD Matrix for $\boldsymbol{\alpha = 0.2}$ Row Sum Matrix

$$\begin{bmatrix} 1 & -1 & 1-1 & 1 & 1 \\ 0 & 0 & 0\ 0 & 1 & 0 \\ -1 & 0 & 0\ 1 & -1 & -1 \end{bmatrix} \qquad \begin{bmatrix} 2 \\ 1 \\ 0 \end{bmatrix}$$

**Table 3.6** Row sum of RTD Matrix for alpha = 0.2

| Age Group | 18-22 | 23-27 | 28-32 |
|---|---|---|---|
| Row Sum | 2 | 1 | 0 |

The Graphical representation of the RTD Matrix at alpha = 0.2

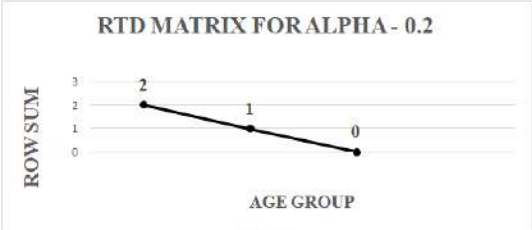



Figure 3.3

The RTD Matrix for $\boldsymbol{\alpha = 0.25}$

$$\begin{bmatrix} 1 & -1 & 1 & -1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ -1 & 1 & -1 & 1 & -1 & -1 \end{bmatrix}$$

Row Sum Matrix

$$\begin{bmatrix} 1 \\ 1 \\ -2 \end{bmatrix}$$

**Table 3.7** row sum of RTD Matrix for alpha = 0.25

| Age Group | 18-22 | 23-27 | 28-32 |
|---|---|---|---|
| Row Sum | 1 | 1 | -2 |

The Graphical representation of the RTD Matrix at alpha = 0.25

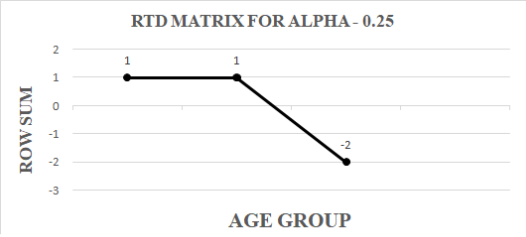



Figure 3.4

Obtain the combined effective time dependent data matrix and the corresponding row sum by adding all the ATD Matrix.

CETD Matrix

$$\begin{bmatrix} 4 & -4 & 4 & -4 & 2 & 2 \\ 0 & 0 & 0 & 0 & 4 & 0 \\ -4 & 3 & -2 & 2 & -4 & -4 \end{bmatrix}$$

Row Sum Matrix

$$\begin{bmatrix} 4 \\ 4 \\ -9 \end{bmatrix}$$

**Table 8** Row sum of CETD Matrix

| Age Group | 18-22 | 23-27 | 28-32 |
|---|---|---|---|
| Row Sum | 4 | 4 | -9 |

The Graphical representation of the CETD Matrix

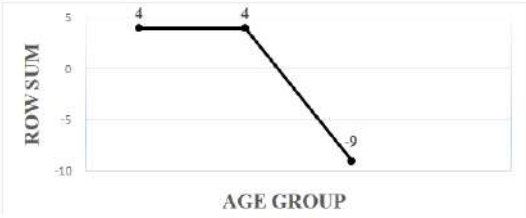



Figure 3.5

## RESULTS AND DISCUSSION

From the observation of the above graph and CETD matrix shows that the age group 18-22 and 23-27 chance to clear the TNPSC exam in first attempt. And it shows that the age group 28-32 was more concentrate to crack the examination. The results of the fuzzy matrix model gave the exact result as that obtained experimentally.

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**RESEARCH ARTICLE**

# Standardization of Swarnavanga with Application of Namboori Phased Spot Test

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## ABSTRACT

*Rasashastra*, an ancient Indian alchemical science imparts knowledge about different formulations which are designed using herbs, minerals and metals. The standardization of these medicines is an important area of research and development in *Rasashastra*. Scientists are constantly developing new and improved methods to standardize these medicines, ensuring that patients have access to safe, effective, and high-quality products.We have classical parameters for assessment of *Rasa* preparations but to update the standards many other parameters are developed. The crucial Namboori Phased Spot Test within *Rasashastra* allows practitioners to assess the authenticity and purity of these substances, ensuring the efficacy and safety of *Rasa Ausadhies***.** Namboori Phased Spot Test(NPST) is a qualitative chemical test for heavy metals. The test is based on the reduction of iodine to iodide by heavy metals in the presence of acid.A sample solution is dropped onto potassium iodide starch paper and observed for a colour change at three time intervals. The colour change depends on the specific heavy metal present and the time interval at which it occurs.The NPST is a simple and reliable test for detecting heavy metals in a variety of samples. It is observed that interaction of *Swarnavanga* with their respective reagents exhibits chromatographic presentation in form of dark brown and other relevant colours appearance and faded gradually which confirms the genuinely of preparation.Similarity seen between observed data and available standard data. The colour and pattern of the spots that appear on the filter paper gives confirmation regarding identification of the authenticity of the *Swarnavanga*.NPST is easy-to-perform test that should be use to identify and assess the quality of *bhasmas* and *sinduras* in a routine. In *AyurvedRasashastra*, this small effort aims to update and enrich the available data regarding the standardization of *Swarnavanga,* making it a valuable tool for Ayurvedic practitioners and researchers.

**Keyword:** *Swarnavanga*, NPST, *Rasashashtra*, *Ayurved*, Standardization.

## INTRODUCTION

*Rasashastra* is branch of an ancient Indian alchemical science, holds immense importance in the realm of *Ayurved*medicine. In an era where traditional medicines are gaining global recognition, the bench side but reliable techniques like the Namboori Phased Spot Test[1] provides a standardized and reliable method for evaluating the quality of medicines derived from metals and minerals.Namboori Phased Spot Test is based on principles of chromatography technique and is crucial for quality assessment of metallic and mineral medicines. This test allows Students, Researchers, Practitioners to assess the authenticity and purity of these substances, ensuring the efficacy and safety of *Rasousadhies*. By preserving the integrity of these ancient practices, *Rasashastra* continues to contribute significantly to the quality assurance of Ayurvedic medicines, promoting health and well-being worldwide.

**Aim:** To assess quality of *Swarnavanga* by Namboori Phased Spot Test

## MATERIALS AND METHODS [2],[3]:

**Materials:**

- 10% Pot iodide paper
- Whatman filter paper
- Solution of 5N $HNO_3$
- Aqua regia
- Distilled water
- Sample – *Swarnavanga*[4]
- Test tubes
- Test tube stand
- Dropper

**Methods:**

- Namboori Phased Spot Test ( NPST )

**Procedure:**

**Preparation of reacting papers**

A solution was prepared by mixing 2 gm Potassium iodide with 20ml distilled water. Subsequently, a Whatman filter paper was immersed in it and then kept on a dry glass sheet to dry.

**Preparation of Solution for *Vanga* Group**

250mg sample was taken into test tubes and 0.5 ml 5N $HNO_3$added as reagent. Test tubes were stirred and heated for a minute then kept undisturbed for 72 hours. After 72 hrs, solution prepared with 5N $HNO_3$ was dropped with the help of dropper on 10% potassium iodide paper and observed in three intervals of time i.e. 0-5 min,5min-20min and 20 min-24 hrs for 1$^{st}$ , 2$^{nd}$ and 3$^{rd}$ phase respectively. Same procedure was conducted with reagent 0.5 ml aqua regia and kept undisturbed for 24 hours. After 24 hrs, solutions prepared with 0.5 ml aqua regia was dropped with the dropper on 10% potassium iodide paper and observation done in three time intervals i.e. 0-5 minutes, 5min-20min and 20 min-24 hours for 1$^{st}$ , 2$^{nd}$ and 3$^{rd}$ phase respectively.

**Preparation of Solution for *Parada* Group**

Similar procedure was carried out for *Parada* group with reagent 0.5ml 5N $HNO_3$ and 0.5ml Distilled water . After 48 hrs, solution prepared with 0.5ml 5N $HNO_3$ and 0.5ml Distilled water each was dropped using dropper on 10%

potassium iodide paper and observed in three intervals of time, 1st phase occurred at 0-5 minutes,2nd phase occurred at 5minutes-20minutes, 3rd phase ocuuredat 20 minutes-24 hours.

## RESULT AND DISCUSSION

For *Vanga* group[Table 1], $HNO_3$ and aqua regia reagents were used. With $HNO_3$, it shows dark brown central spot surrounding by moderate and light brown circle in its 1st phase followed by white space between brown spot and brown periphery having dull margin in 2nd phase then white space reduces and brown periphery expanded to central area in 3rd phase.With aqua regia, it shows dark brown central spot surrounded by light brown circle with clear margin in 1st phase followed by white space between moderate brown central spot and brown periphery having dull margin in 2nd phase and later on in 3rd phase, light central spot and moderate brown periphery with faded margin on outer area.

For *Parada* group[Table 1], $HNO_3$ and distilled water used as reagent.With $HNO_3$, dark brown central spot surrounding by moderate brown circle in 1st phase followed by white space between moderate brown colored central spot and dark brown colored periphery in 2nd phase. In 3rd phase, dull orange internal margin of brown periphery. With distilled water, dull blue space between golden yellow central spot and dull orange periphery in 1st phase followed by central spot disappeared with faded dull orange periphery in 2nd phase. In 3rd phase, periphery area also disappears.

*Rasashastra* is a branch of Ayurvedic science that deals with preparation of herbo-mineral compounds. Herbo-mineral medicines have been used in *Ayurved* for centuries to treat a wide range of diseases and conditions.The standardization of herbo-mineral medicines is important to ensure their safety, efficacy and quality. Standardization involves developing and implementing quality control measures at all stages of the manufacturing process, from the procurement of raw materials to the finished product. The standardization of herbo-mineral medicines is a complex process particularly important in *Rasashastra*, as many of the ingredients used are toxic and must be carefully processed to make them safe for human consumption.

Namboori Phased Spot Test (NPST) is a qualitative analytical test developed by Dr.Namboori Hanumantha Rao in 1970. It is used to identify and assess the quality of *bhasma* and *sindura*, which are herbo-mineral preparations used in Ayurveda. The test is based on the chromatography principle, which is a technique used to separate and identify the components of a mixture. When a drop of a clear solution of a *bhasma* or *sindhura* on Whatman paper impregnated with a suitable reagent, it gives a spot with a series of changes in colour and pattern. The colour and pattern of the spots that appear on the filter paper can be used to identify the authenticity of the sample.

Standardization of *Rasa* preparation is essential need in today's era. The Namboori PhasedSpot Test (NPST) is easy-to-perform test that must be used to identify and assessment of the quality of *bhasma* and *sindura kalpana*, which are Ayurvedic formulations. The test belongs to principles of chromatography and involves observing colour and pattern produced by a chemical reaction between the test sample and a reagent. The NPST is a valuable tool for Ayurvedic practitioners and researchers as it can help to ensure the quality along with consistency for Ayurvedic preparations.

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**Table 1: *Vanga* group**

| Reagent | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| **$HNO_3$** | Dark Brown central spot surrounding by moderate and light brown circle | White space between Brown spot and brown periphery having dull margin | White space reduces and brown periphery expanded to central area |
| **Aqua regia** | Dark Brown central spot surrounded by light brown circle with clear margin | White space between Moderate brown central spot and brown periphery having dull margin | Light central spot And moderate brown periphery with faded margin on outer area |

**Table 2:*Parada* group**

| Reagent | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| **$HNO_3$** | Dark Brown central spot surrounding by moderate brown circle | White space between Moderate brown central spot and dark brown periphery | Dull orange Internal margin of brown periphery |
| **Distilled water** | Dull blue space between Golden yellow central spot and dull orange periphery | Central spot disappeared with faded dull orange periphery | Periphery area also disappears |

Reagent Phase 1 Phase 2 Phase 3

$HNO_3$

Aqua Regia

Figure 1. Vanga Group

| Reagent | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| $HNO_3$ | | | |
| Distilled water | | | |

Figure 2. Parada Group

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**RESEARCH ARTICLE**

# A Probabilistic Machine Learning based Sentiment Analysis Approach for Predicting Customer Reviews

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## ABSTRACT

It is crucial for organizations to determine the characteristics and sentiment of customer evaluations, usually through the use of opinion mining and sentiment analysis. IT systems can utilize natural language processing for a range of functions, such as generating summaries of evaluations, classifying reviews into categories, and performing other real-time applications. Sentiment analysis holds great potential for application in real-time business models. Consumer testimonials serve as an excellent means for companies to obtain feedback. Nevertheless, the process of personally examining a substantial amount of evaluations can be laborious and time-consuming. Sentiment analysis streamlines this procedure, offering a rapid and scalable method to comprehend the entire sentiment sent by clients. Sentiment research aids organizations in identifying favorable client experiences. By discerning favorable sentiments in evaluations, organizations can identify the specific components of their products or services that are highly regarded by customers. This information is vital for strengthening and advocating favorable attributes. Due to the lack of obvious boundaries in classifying tweets or reviews as positive, negative, or neutral, a probabilistic technique using the Deep Bayesian Classifier has been suggested to

determine the sentiment of customer feedback. Evidence demonstrates that the proposed methodology attains higher classification accuracy compared to prior research in this domain.

**Keywords:** Data Mining, Customer Review, Sentiment Analysis, Machine Learning, Bayesian Classifier, Classification Accuracy.

## INTRODUCTION

Customer sentiment analysis relies heavily on machine learning, which gives companies an effective tool for understanding and reacting to customer feedback. In a day of digital communication and information overload, it is critical to comprehend the feelings that customers are expressing. This paper examines the several factors that make machine learning essential for efficient customer sentiment analysis [1]. However, it is difficult to extract user sentiment from vast and complicated data sets. This is to make sure that the implicit meaning must be accurately deduced and that the context (semantics) is considered before drawing any judgments. To further separate the valuable information from the raw data, precise data pre-processing must be mandated. Sentiment analysis is crucial since user attitudes significantly affect a number of characteristics and domains. Machine learning's ability to manage enormous amounts of consumer input is one of the main reasons it is crucial for sentiment analysis. Businesses now receive a dizzying quantity of information from several sources, including surveys, emails, social media, and online reviews. This scale is beyond the capabilities of traditional methodologies; in contrast, machine learning models process massive datasets effectively, allowing organisations to analyse feelings more broadly [2].

Sentiment analysis faces a great deal of difficulty because of how intricate human language is. Natural language processing (NLP) in particular, falls under machine learning is excellent at identifying intricate patterns, contextual subtleties, and minute differences in consumer reactions. Beyond crude positive or negative classifications, this capacity to interpret linguistic nuances enables a more precise and sophisticated sentiment analysis. Consumer perceptions are subject to alter due to new product offers, shifting trends, and outside events. By continuously learning from fresh data, machine learning models demonstrate adaptability. This flexibility guarantees that sentiment research is current and representative of the customer landscape, allowing organisations to react quickly to shifting emotions and make well-informed decisions.Personalised sentiment analysis catered to certain sectors and circumstances is made possible by machine learning. Businesses can tailor sentiment analysis tools to meet their specific needs by training models on language specific to their industry. By taking a customised approach, sentiment research becomes more accurate and companies pay attention to the things that are most important to their customers [3].

A generic process architecture for sentiment analysis through the knowledge discovery process in depicted in figure 1. Automatic feature extraction, key phrase recognition, and word associations with positive or negative sentiments are areas where machine learning can be employed due to the enormity of data being analyzed.. This feature gives companies the ability to identify particular features of goods or services that affect how customers perceive them, giving them insightful information for future development.

## LITERATURE REVIEW

The literature survey of various scholars related to the review is as follows:- Zhao et al. [1] proposed a multimodal sentiment analysis technique built on the multimodal sentiment analysis technique, which can help individuals make better judgments by obtaining more emotional information sources. According to this paper's experimental findings, CNN-SVM has the greatest recognition rate. 93.5%, respectively. Dhyani et al. in [2] author proposed for sentiment analysis, a brand-new intuitionistic fuzzy inference system (IFIS). Through the suggested IFIS, the study article uses sentiment analysis of tweets to predict the personality attribute features of the tweeter. Utilising TextBlob in Google

Collaboration, the Natural Language Processing Toolkit (NLTK) was utilised to examine Twitter data for subjectivity and polarity, with the goal of predicting the positivity score through the suggested innovative IFIS.Vohra et al. [3] proposed To get outstanding outcomes on the dataset, the model makes use of numerous convolution and max pooling layers, dropout operation, and dense layers with ReLU and sigmoid activations. Furthermore, a comparison is made between the proposed model's performance and that of many common classifiers, including Random Forest, Naive Bayes, Support Vector Machine (SVM), and Decision Tree. According to the findings, the suggested CNN with FastText word embeddings performs better than other classifiers on the provided dataset, with an accuracy of 0.925969... This classification reveals, nearly 55% of the tweets are found to show affirmation, about 25% show a negative disposition, and 21.09% have neutral sentiments Phan et al. [4] proposed a model which comprises the subsequent actions: First, BERT is used to turn words in sentences into vectors. Secondly, BiLSTM over word vectors is used to produce the contextualised word representations. Third, across the contextualised word representations, important characteristics are retrieved and represented using the GCN model with several convolutional layers. Lastly, the CNN model is applied to the feature vectors in order to classify the feelings at the aspect level. Tests conducted on three reference datasets demonstrate that the performance of the prior context-based GCN techniques for ALSA has been enhanced by the proposed model.

Obiedat et al. [5] proposed a combination approach to address the unbalanced data issue that combines the Support Vector Machine (SVM) algorithm with Particle Swarm Optimisation (PSO) and several oversampling approaches. SVM is used as a machine learning classification approach to optimize the dataset, which includes various reviews from a number of Jordanian restaurants, in order to anticipate the sentiments of reviews. Information was gathered from Jeeran, a popular Arabic review social network. The feature weights are optimized using the PSO approach. Vashishtha et al. [6] proposed a combination approach to address the unbalanced data issue that combines the Support Vector Machine (SVM) algorithm with Particle Swarm Optimisation (PSO) and several oversampling approaches. SVM is used as a machine learning classification approach to optimise the dataset, which includes various reviews from a number of Jordanian restaurants, in order to anticipate the sentiments of reviews. Information was gathered from Jeeran, a popular Arabic review social network. The feature weights are optimised using the PSO approach.

Saha et al. [7] utilised a variety of machine learning methods to analyse sentiment. On the linguistic characteristics, algorithms including Multilayer Perceptron (MP), Random Forest (RF), Decision Tree (DT), and Support Vector Machine (SVM) were used. For every classifier, the authors have calculated the precision, recall, accuracy, F-measure, and ROC values. Random Forest scored better than the other classifiers, successfully classifying nearly 61% of the instances. Such sentiment analysis, in our opinion, on a particular class of texts might inspire more research into our grasp of natural language. Estrada et al. [8] The authors compared various sentiment analysis classifiers within an Intelligent Learning Environment (ILE-Java) to categorize educational opinions. Three different methodologies were employed: machine learning, deep learning, and an evolutionary technique called EvoMSA. The researchers curated two datasets focusing on programming languages, encompassing students' sentiments regarding professors, tests, assignments, and academic projects. SentiTEXT comprises polarity labels (positive and negative), while eduSERE includes labels related to learning-oriented emotions (positive and negative), such as engaged, thrilled, bored, and annoyed.

Rahat et al. [9] proposed a technique to analyze the sentiment polarity of airline review dataset based on a numerical transformation of the data prior analysis. The removal of special characters such as @ removal etc. was the first step in the pre-processing stage. It could be inferred from the experimental results that the SVM outperformed the Naïve Bayes for the airline dataset. Hasanli et al. [10] A strategy outlining the steps for gathering, cleaning, and annotating tweets in Azerbaijani has been developed for sentiment analysis of the language. Based on bag-of-words models, machine learning methods such as SVM, Naïve Bayes, and Linear Regression were used to determine sentiment polarity. Other Turkish languages can easily adopt our transferable and easily implementable data processing and categorization approach. By comparing the outcomes of several machine learning algorithms, the best parameters for tweet categorization were found..

# METHODOLOGY

**Sentiment Analysis using Machine Learning**

When it comes to expressing and ranking whether a text reflects good, neutral, or negative emotions, machine learning algorithms are frequently quite useful. There are two categories of extensions for machine learning, such as unsupervised methods. A labelled dataset is used by the supervised algorithm, and every training text is created with positive sentiments. On the other hand, unsupervised readings consist of happy feelings and unprocessed data without any writing.. The commonly used machine learning models used are [11].

**Convolutional Neural Network (CNN):** CNN's basic architecture is made up of layers that handle incoming data in a hierarchical fashion. The main parts are fully connected layers, pooling layers, and convolutional layers. By using filters or kernels to the input data, convolutional layers extract features by identifying local patterns and details. In order for CNNs to learn hierarchical representations from unprocessed input, these layers are essential. Convolutional layers carry out the vital function of extracting features. Sliding a filter over the input data and calculating the dot product at each place comprise the convolution procedure. Activation functions add non-linearity to the network after the convolutional process. ReLU (Rectified Linear Unit), a popular activation function, introduces non-linearities by thresholding the output, allowing the model to learn more intricate feature correlations [12].

**Support Vector Machine (SVM):** One potent class of machine learning techniques used for both regression and classification applications is Support Vector Machines (SVMs). They have become well-known for their reliable operation and adaptability in a variety of fields. The goal behind Support Vector Machines (SVMs) is to identify the optimal hyperplane for separating data points into distinct groups. By acting as the decision boundary, this hyperplane maximises the margin between the classes. SVMs work well in high-dimensional environments, where they are useful for jobs requiring a large number of input features. SVMs are unique in that they prioritise maximising the gap between each class's nearest data points and the decision border. The distance between the closest data point and the hyperplane is known as the margin [13].

**Hyperplane in SVM:** The data points that are closest to the decision boundary or hyperplane are known as support vectors. In order to define the margin and, by extension, the decision boundary, these points are essential. These support vectors are what give support vector machines (SVMs) their moniker because they help find the best possible hyperplane. With non linear separable datasets depending on multiple variables, the SVM employs a mult-dimensional hyperplane (hyperplane with working with multiple variables) making data separable. Polynomial, sigmoid, and radial basis function (RBF) are examples of common kernel functions. Optimising a cost function that takes into account the margin size and classification accuracy is necessary for training a support vector machine. The goal of the optimisation method is to identify the ideal hyperplane [14].

**Bayesian Approach**

Probabilistic graphical models known as Bayesian Networks, or BayesNets, use Bayesian probability to depict and infer relationships between variables. Regarding sentiment classification, BayesNets provide a logical and comprehensible method. This paper explores the use of BayesNets in sentiment classification, emphasising the advantages and drawbacks of this method for identifying emotional overtones in textual data. Determining the sentiment expressed in a text and classifying it as good, negative, or neutral is known as sentiment classification. A probabilistic modelling framework that is well-suited to the ambiguity contained in language is offered by BayesNets, which enables the capture of dependencies between words or phrases and the nuanced depiction of sentiment [15]. With directed acyclic graphs (DAGs), Bayesian networks depict conditional dependencies between variables. Nodes in the network can represent words or features in the context of sentiment categorization, while edges show conditional dependencies. Because of this structure, BayesNets can accurately represent how the presence or absence of specific words affects the likelihood of a given feeling. In a BayesNet, each node has associated probabilities that indicate the chance that a variable will take on a specific value based on the values of its

parent nodes. These probabilities, which indicate the possibility of noticing particular words or features given the sentiment indicated in the text, can be learned from training data in sentiment classification. Sentiment analysis requires the ability to handle uncertainty and variability, which BayesNets naturally provide. It's common for language to be vague, and there are many methods to convey feelings. Because BayesNets incorporate prior probabilities and adapt their beliefs based on mounting information, they facilitate the modelling of uncertainty. They are therefore capable of managing a wide range of language manifestations of emotion. Prior knowledge can be included with BayesNets, which is especially helpful for sentiment categorization. An enhanced and contextually aware sentiment analysis can be achieved by encoding into the network prior knowledge about words or phrases that strongly suggest a particular sentiment [16].

**Algorithm**

As the customer review texts may have overlapping tags or tokens, hence a probabilistic Bayes Classifier has been proposed.As sentiments do not possess a particular decision boundary (fixed), hence a probabilistic approach happens to be more effective which can be done employing the Deep Bayes Net whose classification depends on the following relation:

$$\boldsymbol{P}\left(\frac{X}{X_i,k_1,k_2,M}\right)=\frac{P\left(\frac{X_i}{X,k_2,M}\right)P\left(\frac{X_i}{k_1,M}\right)}{P\left(\frac{X}{k_1,k_2,M}\right)} \quad \textbf{(1)}$$

Here,

$P$represents probability.

$X_i$represents weights and bias vectors (combined).

$X$represents the data to be used for the purpose of training.

$M$represents data units (neurons) in network.

$k_1$ $and$ $k_2$represents the term responsible for penalty based regularization.

$\rho=\frac{k_1}{k_2}$ is often considered the regularization factor which is acted upon the objective function (J) to me optimized based on the training dataset, and renders the regularized cost function:

$$\boldsymbol{F(w)=\mu w^T w+v[\frac{1}{n}\sum_{i=1}^{n}(p_i-a_i)^2]} \quad \textbf{(2)}$$

If $(\pi \ll v)$:errors in training are typically rendered low.

else if $(\pi \geq v)$:errors are typically rendered high needing a weight reduction or Penalty.

The proposed algorithm is presented next:

***Start***

*{*

***Step.1*** *Obtain annotated dataset.*

***Step.2*** *Divide the data into a ratio of 70:30 as training and testing data samples.*

***Step.3*** *Define match token data length (n) and*

$for\ i=1{:}n$

$Search\ (token == match\ text)$

*end*

**Step.4** Design a neural network with multiple hidden layers.

**Step.5** Initialize training with random weights.

***Step. 6*** Train models with training data and updated weights based on the back propagation rule as:

$$\boldsymbol{w_{k+1} = w_k - \left[J_k J_k^T + \mu I\right]^{-1} J_k^T e_k} \quad \textbf{(3)}$$

**Step.7** if (Cost Function J stabilizes over multiple iterations)
Truncate
else if (iterations==max. iterations defined)
Truncate
else
{
Apply data and update (w, b)
Feedback (e)
}

**Step.8** Calculate error% and Classification Accuracy
**Stop**
}

The performance parameters used for evaluation of the algorithm is the accuracy % which is computed as:

$$\boldsymbol{Accuracy\% = 100 - error\%} \quad \textbf{(4)}$$

## EXPERIMENTAL RESULTS

The experimental results have been presented next. The dataset has been taken from Kaggle, for Amazon Product Reviews.
(https://www.kaggle.com/datasets/tarkkaanko/amazon?resource=download)
Out of the 4916 samples, the training and testing ratio has been selected as 70:30 making the testing samples as 1475 samples. From table 2, it can be clearly observed that the proposed work attains much higher classification accuracy employing the Regularization based Deep BayesNet approach due to a probabilistic classification approach. Moreover, a grouped word match also aids the classification process.

## CONCLUSION

To summarize, Sentiment analysis has emerged as an essential tool for business objectives, providing significant insights into user behaviors and preferences. Various methods and algorithms within Natural Language Processing (NLP) are used to achieve a thorough comprehension by applying sentiment analyzers. This study offers a comprehensive examination of several datasets and research studies that utilize different machine learning methods for sentiment analysis. Machine learning-based sentiment analysis has demonstrated its efficacy and adaptability as a beneficial tool for obtaining important insights from textual data. The research that were evaluated demonstrated a wide variety of strategies, including both traditional methods like Random Forest and Word2Vec, as well as more modern approaches like Recurrent Neural Networks (RNN). These techniques have shown a high level of accuracy in determining sentiment in many areas. There is great potential for further progress in the field of video sentiment analysis, namely in the areas of extracting features from frames and applying machine learning methods. In summary, the research highlights the importance of machine learning in uncovering patterns of sentiment, which helps us gain a more profound comprehension of user feelings across various applications and domains. The study introduces an enhanced Bayesian Network for analyzing the Amazon Product Review dataset. The model achieves a

Mean Squared Error (MSE) of 0.95973 and a Mean Absolute Error (MAE) of 0.67, surpassing the accuracy of current baseline methods.

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**Table 1. Summary of Previous Work**

| Author | Method / Algorithm | Dataset | Research gap | Parameter |
|---|---|---|---|---|
| Zhao et al. [1], 2023 | CNN-SVM | eNTRAFACE'05, RML, and AFEW6.0 | No data pre-processing employed.<br><br>Amalgamation of baseline techniques such as CNN-RNN and SVM often leads to quick saturation of performance as more training data is added. (Inherent to SVM) | Accuracy of 93.5 % |
| Dhyani et al [2], 2022 | Intuitionistic Fuzzy Inference System (IFIS) | Kaggle<br><br>(Twitter Dataset) | Fuzzy Inference System (FIS) used for sentiment analysis. Typically fuzzy systems also suffer from performance saturation. A better option is the Adaptive Neuro-Fuzzy Inference System (ANFIS). | RMSE of 0.3078 |
| Vohra et al. [3], 2022 | CNN | Kaggle<br><br>(Twitter Dataset) | CNN is often prone to overfitting and vanishing gradient. No analysis pertaining to overfitting and vanishing gradient performed. | Accuracy of 92.59 % |
| Phan et al. [4], 2022 | Graph Convolutional Neural Networks (GCN) | Kaggle<br><br>(Twitter Dataset) | The Graph Convolutional Neural Networks (GCN) used for The CNN model is used to classify aspect-level sentiments instead of feature vectors. CNN often tend to exhibit overfitting.<br><br>Moreover, probabilistic approaches may tiled higher accuracy for aspect level classification. | Accuracy of 85.25% |
| Obeidat et al. [5],<br><br>2022 | PSO-SVM | Kaggle | The PSO-Optimized SVM approach has an inherent data saturation probability. Mo word-frequency analysis has been used. | Accuracy of 89% |
| Vashishtha et al. [6], 2021 | Adaptive Neuro Fuzzy Inference Systems (ANFIS) | Kaggle<br><br>(SentiwirdNet) | ANFIS typically exhibits early performance saturation. No group or bag of words match performed which may increase prediction accuracy. | Best case RMSE of 0.36 |
| Saha et al. [7],<br><br>2021 | Decision Trees (DT) | Kaggle | No data pre-processing. Frequency analysis of words not employed. Decision trees typically does not match the performance of neural networks and deep neural networks. | Accuracy of 60.5395% |
| Estrada et al. [8], 2020 | CNN-LSTM hybrid | SentiWord | Both the CNN and LSTM are prone to overfitting. They also suffer from the vanishing gradient problem while training with large training dataset corpus. No probabilistic approach explored for context level classification. | Accuracy of 93% |

**Table.2 Summary of Results**

| S.No | Parameter | Value |
|---|---|---|
| 1 | Dataset | Amazon Product Review Dataset |
| 2 | ML Category | Supervised |
| 3 | Algorithm | Bayesian Optimized Neural Network |
| 4 | Iterations to Convergence | 45 |
| 5 | Mean Squared Error (MSE) | 0.95973 |
| 6 | Mean Absolute Error (MAE) | 0.67 |
| 7 | Accuracy % | 99.34 |

**Table 2. Comparison with Previous Work**

| S.No. | Author | Accuracy (%) |
|---|---|---|
| 1 | Zhao et al. [1] | 93.5 |
| 2 | Vohra et a. [3] | 92.59 |
| 3 | Phan et al. [4] | 82.5 |
| 4 | Obiedat et al. [5] | 89 |
| 5 | Saha et al. [7] | 60.5395 |
| 6 | Estrada et al. [8] | 93 |
| **7** | Proposed Work | 99.34% |

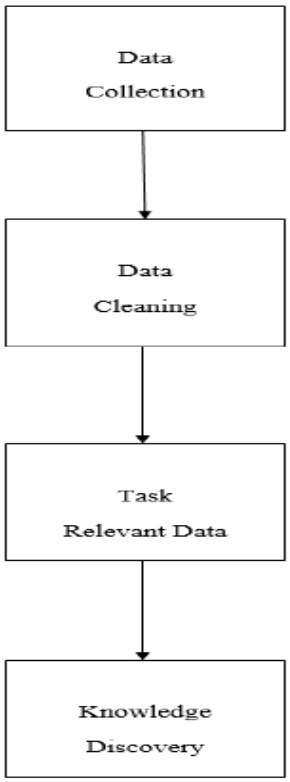
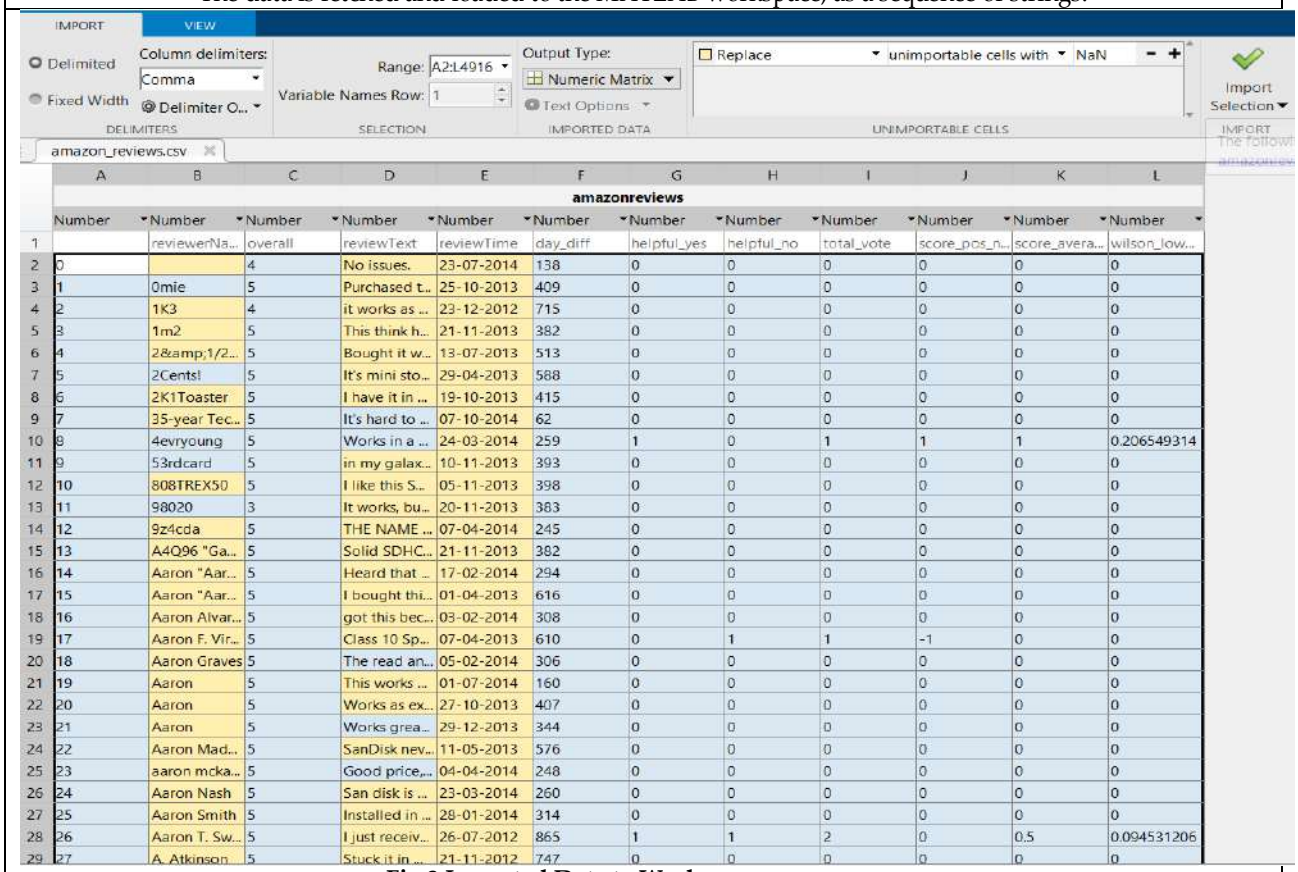



**Fig.1 Steps for Sentiment Analysis**

| 1 | | reviewerName | overall | reviewText | reviewTime | day_diff | helpful_ye | helpful_no | total_vote | score_pos | score_ave | wilson_lower_bound |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0 | | 4 | No issues. | 23-07-2014 | 138 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1 | 0mie | 5 | Purchased this for my device, it worked as | 25-10-2013 | 409 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 2 | 1K3 | 4 | it works as expected. I should have sprung | 23-12-2012 | 715 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3 | 1m2 | 5 | This think has worked out great.Had a diff. | 21-11-2013 | 382 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 4 | 2&1/2Men | 5 | Bought it with Retail Packaging, arrived leg | 13-07-2013 | 513 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 5 | 2Cents! | 5 | It's mini storage. It doesn't do anything els | 29-04-2013 | 588 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 6 | 2K1Toaster | 5 | I have it in my phone and it never skips a b | 19-10-2013 | 415 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 7 | 35-year Technology ( | 5 | It's hard to believe how affordable digital h | 07-10-2014 | 62 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 8 | 4evryoung | 5 | Works in a HTC Rezound. Was running shc | 24-03-2014 | 259 | 1 | 0 | 1 | 1 | 1 | 0.206549 |
| 11 | 9 | 53rdcard | 5 | in my galaxy s4, super fast card, and am to | 10-11-2013 | 393 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 10 | 808TREX50 | 5 | I like this SD Card because it can take musi | 05-11-2013 | 398 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 11 | 98020 | 3 | It works, but file writes are a bit slower tha | 20-11-2013 | 383 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 12 | 9z4cda | 5 | THE NAME OF ITSELF SPEAKS OUT. GO SAN | 07-04-2014 | 245 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 13 | A4Q96 "Gadget Love | 5 | Solid SDHC card that is fast (at reading and | 21-11-2013 | 382 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 14 | Aaron "Aaron" | 5 | Heard that the card's write speed is insuffi | 17-02-2014 | 294 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 15 | Aaron "Aaron" | 5 | I bought this to use with my go pro hero 3 | 01-04-2013 | 616 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 16 | Aaron Alvarez | 5 | got this because i had a 2 GB one that fille | 03-02-2014 | 308 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 17 | Aaron F. Virginie | 5 | Class 10 Speed Rating for Seamless Full HD | 07-04-2013 | 610 | 0 | 1 | 1 | -1 | 0 | 0 |
| 20 | 18 | Aaron Graves | 5 | The read and write speeds are better than | 05-02-2014 | 306 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 19 | Aaron | 5 | This works with the NL1520. No video stu | 01-07-2014 | 160 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 20 | Aaron | 5 | Works as expected. High transfer speed. I | 27-10-2013 | 407 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 21 | Aaron | 5 | Works great in a Samsung Galaxy S3. Form | 29-12-2013 | 344 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 22 | Aaron Madden | 5 | SanDisk never disappoints. As always SanD | 11-05-2013 | 576 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 23 | aaron mckaig | 5 | Good price, works flawless in my Samsung | 04-04-2014 | 248 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 24 | Aaron Nash | 5 | San disk is hard to beat. You will pay more | 23-03-2014 | 260 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 25 | Aaron Smith | 5 | Installed in my Blackberry Q10 SQN100-1 a | 28-01-2014 | 314 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 26 | Aaron T. Swain | 5 | I just received my card, it is the class 10 64 | 26-07-2012 | 865 | 1 | 1 | 2 | 0 | 0.5 | 0.094531 |

**Fig.2 Raw Data.**

The data is fetched and loaded to the MATLAB workspace, as a sequence of strings.




| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number | Number | Number | Number | Number | Number | Number | Number | Number | Number | Number | Number |
| 1 | | reviewerNa... | overall | reviewText | reviewTime | day_diff | helpful_yes | helpful_no | total_vote | score_pos_n... | score_avera... | wilson_low... |
| 2 | 0 | | 4 | No issues. | 23-07-2014 | 138 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1 | 0mie | 5 | Purchased t... | 25-10-2013 | 409 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 2 | 1K3 | 4 | it works as ... | 23-12-2012 | 715 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3 | 1m2 | 5 | This think h... | 21-11-2013 | 382 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 4 | 2&1/2... | 5 | Bought it w... | 13-07-2013 | 513 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 5 | 2Cents! | 5 | It's mini sto... | 29-04-2013 | 588 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 6 | 2K1Toaster | 5 | I have it in ... | 19-10-2013 | 415 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 7 | 35-year Tec... | 5 | It's hard to ... | 07-10-2014 | 62 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 8 | 4evryoung | 5 | Works in a ... | 24-03-2014 | 259 | 1 | 0 | 1 | 1 | 1 | 0.206549314 |
| 11 | 9 | 53rdcard | 5 | in my galax... | 10-11-2013 | 393 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 10 | 808TREX50 | 5 | I like this S... | 05-11-2013 | 398 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 11 | 98020 | 3 | It works, bu... | 20-11-2013 | 383 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 12 | 9z4cda | 5 | THE NAME ... | 07-04-2014 | 245 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 13 | A4Q96 "Ga... | 5 | Solid SDHC... | 21-11-2013 | 382 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 14 | Aaron "Aar... | 5 | Heard that ... | 17-02-2014 | 294 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 15 | Aaron "Aar... | 5 | I bought thi... | 01-04-2013 | 616 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 16 | Aaron Alvar... | 5 | got this bec... | 03-02-2014 | 308 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 17 | Aaron F. Vir... | 5 | Class 10 Sp... | 07-04-2013 | 610 | 0 | 1 | 1 | -1 | 0 | 0 |
| 20 | 18 | Aaron Graves | 5 | The read an... | 05-02-2014 | 306 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 19 | Aaron | 5 | This works ... | 01-07-2014 | 160 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 20 | Aaron | 5 | Works as ex... | 27-10-2013 | 407 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 21 | Aaron | 5 | Works grea... | 29-12-2013 | 344 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 22 | Aaron Mad... | 5 | SanDisk nev... | 11-05-2013 | 576 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 23 | aaron mcka... | 5 | Good price,... | 04-04-2014 | 248 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 24 | Aaron Nash | 5 | San disk is ... | 23-03-2014 | 260 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 25 | Aaron Smith | 5 | Installed in ... | 28-01-2014 | 314 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 26 | Aaron T. Sw... | 5 | I just receiv... | 26-07-2012 | 865 | 1 | 1 | 2 | 0 | 0.5 | 0.094531206 |
| 29 | 27 | A. Atkinson | 5 | Stuck it in ... | 21-11-2012 | 747 | 0 | 0 | 0 | 0 | 0 | 0 |

**Fig.3 Imported Data to Workspace**

**Figure 3 shows the raw data being imported to the workspace.**

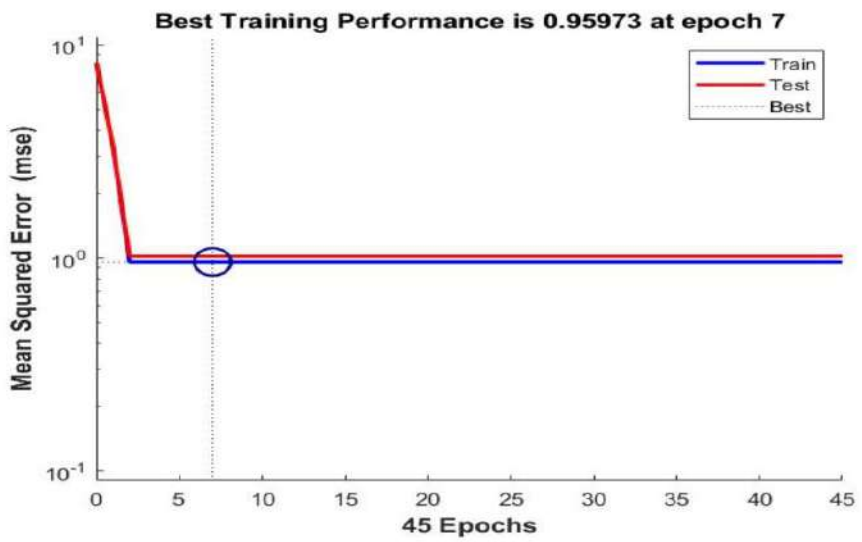



**Fig.4 Convergence of algorithm w.r.t. iterations**

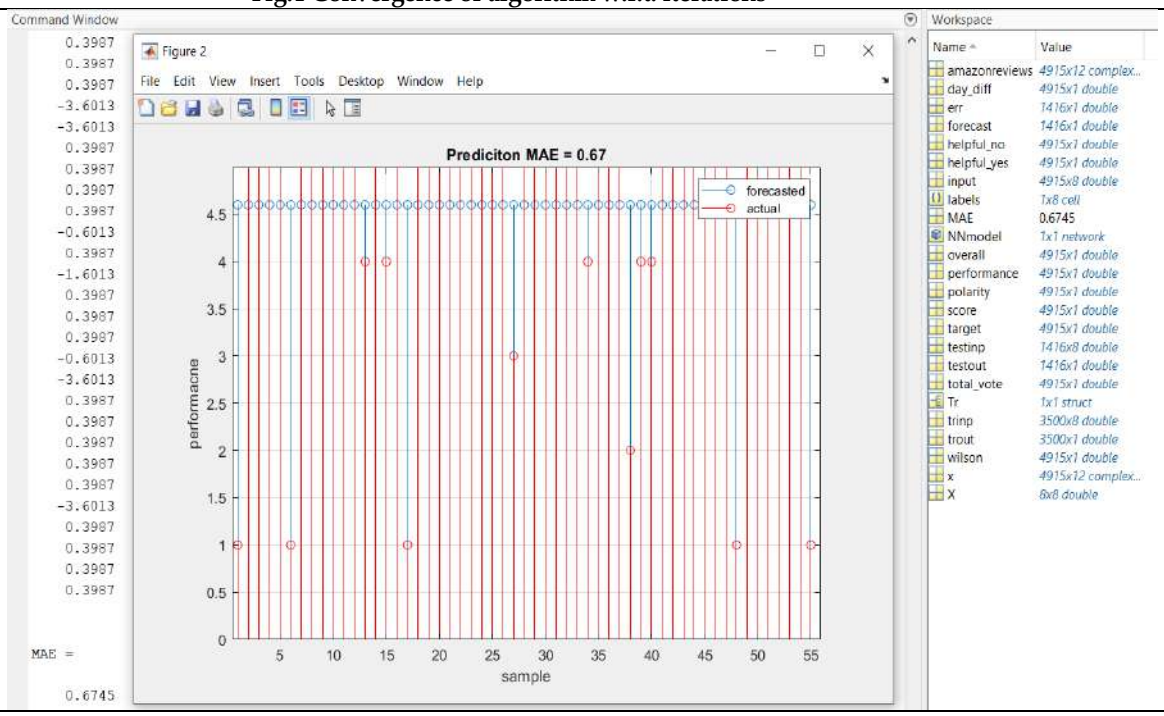



**Fig.5 Command Line Screenshot**

**Figure 5 shows the command line screenshot for mean absolute error.**

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**RESEARCH ARTICLE**

# Katiparinaah and Body Adiposity Index in Non Alcoholic Fatty Liver Disease : A Cross-Sectional Analytical Study

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Cross-sectional analytical study

There is an increasing prevalence of non alcoholic fatty liver disease (NAFLD) in India at an early age due to the unhealthy diet and lifestyle of people. Modern medicine does appreciate the prediction of fat deposition through body mass index (BMI) to predict the metabolic risk, but BMI directly depends upon the weight of the body. Unlike BMI, body adiposity index (BAI) does not depend upon the weight of the body. The concept of proportionate body measurements (*Sama Sharir*) of Ayurveda is associated with good health and long life. The objective of the study was to discriminate the measurement of *Katiparinaah* and body adiposity index in between NAFLDpatients and healthy individuals without fatty liver. Cross-sectional analytical study A total of 153 females of age group 20 to 50 years were registered in two groups; 66 NAFLDfemale patients (case group) and 87 healthy without fatty liver females (control group). Waist circumference, one *anguli* breadth, *Katiparinaah*, and body adiposity index were recorded for the registered subjects after informed consent. The measurement of *Katiparinaah* and body adiposity index is significantly higher in the female patients of NAFLDin comparison to healthy females without fatty liver ($P< 0.05$). The ROC analysis indicates that *Katiparinaah* and body adiposity are moderate to good predictors for predicting NAFLD. *Katiparinaah* >=57.03 *anguli* and body adiposity index of >=32.53 are the cut-off points for the discrimination between healthy non-fatty liver and non-alcoholic fatty liver disease. There is a significant difference in *Katiparinaah* and body adiposity index in the female patients of non-alcoholic fatty liver disease in comparison to healthy female subjects, Females of *Katiparinah* >= 57.03 *anguli* or body adiposity index >=32.53 are at higher risk of developing NAFLD.

**Keywords:** non-alcoholic fatty liver, *Sama Sharir*, *Katiparinaah*, body adiposity index, anthropometry.

## INTRODUCTION

The liver is the most common site for the accumulation of fat because it plays a central role in fat metabolism. Depending upon the cause and amount of accumulation, fatty liver changes may be severe, irreversible, and may result in cell death [1]. Adiposity (excess fat deposition) is the second most common cause of fatty liver and the most common cause of non-alcoholic fatty liver disease (NAFLD). There is an increasing prevalence of NAFLDin India at an early age due to the unhealthy lifestyle of people and it contributes to a large proportion of liver disease burden in the world [2]. The concept of *sama sharir* (proportionate body measurements) of Ayurveda is associated with good health and long life. The scholars of Ayurveda (*Charak, Sushruta, Vagabhat*) have mentioned the reference value of measurements of different parts of the body. [3]-[5]. These reference values give the measurements of a proportionate body in one's finger measurement, called *swa anguli pramana*. Unlike the contemporary reference values of measurement of the body, the *swa anguli* measurement is personalized. A person of proportionate body measurements (*sama sharir*) had a height of 84 *anguli*, *Prabahu* (forearm) 16 *anguli* and *Prapani* 15 *anguli*, *Katiparinaah* (waist circumference) 50 *anguli*. It can be hypothesized that the deviations in the measurement of body parts from the reference value can be considered as disproportionate body and it can lead to diseased condition. *Katiparinaah* indicates the abdominal obesity and its deviation from the reference value can be a predictor of fatty liver disease of adiposity origin (NAFLD) in a very early stage of the disease.

Modern medicine does appreciate the prediction of fat deposition through body mass index (BMI) to predict the metabolic risk and diseases linked to adiposity, but BMI directly depends upon the weight of the person [6] which does not include fat mass only but also the bone, muscle mass, and water content of the person, so the BMI does not give the true picture of adiposity. In such a scenario, it is necessary to make available an alternative method, which can predict the excess fat deposition and the diseases related to it. Body Adiposity Index (BAI) has been presented as a potential alternative to BMI. Unlike BMI, BAI does not depend upon the weight of the body and is calculated by the size of the hip circumference compared to the person's height. Bergman et al. reported that BAI is strongly correlated with adiposity, and in contrast to BMI, BAI is equally good for both genders and differing ethnicities. BAI could directly estimate the percent body fat without the need for further correction for sex or age [7].

The objective of the study was to analyze the difference in the measurement of *Katiparinaah* (*in anguli)* and body adiposity index independently between NAFLDpatients and healthy individuals without fatty liver.

## MATERIALS AND METHODS

Study design- cross-sectional analytical study. The study population for this study was the female patients approaching the hospital for consultation and the healthy females residing in nearby villages. A total of 153 females were registered in the study. Of the total study participants, 66 females were patients of NAFLDin case group and 87 healthy females without fatty liver were registered in control group. (Table 1). Ethical permission was taken from the institute's ethical committee (letter no CBP-IEC/2020/RS 07/MD/28 dated 29/01/2022) and the study was registered in the Clinical trial registry India before the start of enrolment of study participants. It is obvious that the anthropometric measurements of body naturally vary between males and females, so the present study is limited to female study participants only to remove the gender confounder. The study participants were well-versed about the data collection and their privacy. All the study participants were of age group 20 to 50 years, and registered after informed consent, in the year 2022-23. All the 153 participants of both the groups were confirmed for fatty liver and non-fatty liver by abdominal ultrasound. Individuals with a history of alcohol consumption, medications that cause liver damage and steatosis like estrogen, steroids, tetracycline, active or previous infection with hepatitis B and C, and other liver disease, chronic illness (T.B), diabetic patients, malnourished individuals, and individuals under starvation and pregnant women were excluded from the study.

Standard anthropometric measurements (height, hip circumference, waist circumference) were measured by stadiometer, and measuring tape respectively. Four fingers' width of the right hand was measured by a digital vernier caliper of 300 mm scale between the proximal inter-phalangeal joint of the 2nd digit and the distal inter-phalangeal joint of the 5th digit from the dorsal aspect after keeping the hand in supine on the top of a sturdy table. This measurement was divided by four to get one *anguli* (finger) width [8].

All the data was recorded in the Performa along with other demographic data. The *Katiparinaah* in *anguli praman* was calculated by dividing the waist circumference (cm) by one *anguli* width (cm) of the person. Body adiposity index was calculated by the formula:

BAI =Hip circumference in cm/(height (m)$^{1.5}$ -18) [9]

The difference of the *Katiparinaah* and body adiposity index individually among the two groups was analyzed using unpaired t test and the cut-off point of the two variables to predict the NAFLDwas measured by Receiver operating characteristic (ROC).

## RESULT

**Discrimination of *Katiparinaah* between NAFLDand healthy females without fatty liver**

The mean and standard deviation of *Katiparinaah* in control and case group was 53.64 ± 3.61 *anguli*, and 58.52 ± 5.65 *anguli* respectively. The difference of *Katiparinaah* in between case and control group was statistically significant ($P$<0.0001) **(**Table 2). The ROC (Receiver Operating Characteristic) analysis was performed to evaluate the diagnostic accuracy of *Katiparinaah* in detecting Fatty Liver Disease. The area under ROC is a measure of the overall discriminatory ability of *Katiparinah* in distinguishing between individuals with and without fatty liver disease, the area under ROC was found to be 0.768, indicating a reasonably good discriminatory power. The optimum cut-off value (the threshold of *Katiparinaah* level) that provides the best balance between sensitivity and specificity for detecting non-alcoholic fatty liver disease in females was: *Katiparinah* >= 57.03 *anguli* (Table 3)

**Discrimination in Body adiposity index between NAFLDand healthy without fatty liver females**

The mean and standard deviation of body adiposity index in control and case group was 28.5 ± 2.56, and 31.22 ± 5.46 respectively. The difference of body adiposity index was statistically significant in case and control group ($P$<0.0001) (Table 4). The above results indicate that the BAI is a moderate predictor of Fatty Liver Disease in females. A body adiposity index value of 32.53 or higher is the optimal cut-off for detecting the NAFLDin females, achieving a sensitivity of 37.9% and specificity of 97.9%. (Table 5)

**Variation of *Katiparinaah* with fatty liver grades in NAFLDpatients**

The mean and standard deviation of *Katiparinaah* in grade I and grade II was 57.28 ± 5.03 *anguli*, and 62.34 ± 6.01 *anguli* respectively, and the difference of *Katiparinaah* in grade I and grade II fatty liver disease was found statistically significant ($P$=0.0015) (Table 6)

**Variation of body adiposity index with fatty liver grades in NAFLDpatients**

The mean and standard deviation of body adiposity index in grade I and grade II fatty liver was 30.13 ± 4.86, and 34.57 ± 6.09 respectively. Statistically significant difference of body adiposity index was found in grade I and grade II fatty liver disease ($P$=0.0041) (Table 7)

## DISCUSSION

The individuals who are disproportionate by the criteria of *anguli pramana* may have *meda dhatu dushti*, which could be associated with diseases of excess fatty deposition like risk of cardio vascular ischemia, non-alcoholic fatty liver etc. The *Katiparinaah* and body adiposity index are significantly higher in the females of NAFLDin comparison to healthy females without fatty liver ($P$< 0.05). The ROC analysis indicates that *Katiparinaah* and body adiposity are

moderate to good predictors for predicting NAFLD. Females of *Katiparinaah* >=57.03 *anguli* and body adiposity index of >=32.53 are at a higher risk of developing NAFLD. The severity (grade) of fatty liver disease also increases with the increase of *Katiparinaah* and body adiposity index. Previous studies of *anguli praman* and anthropometry have demonstrated that smaller facial structures and shorter neck limbs are associated with higher insulin resistance markers (obesity, adiposity, glycemia, insulin, and HOMA-R, but larger forehead and face were associated with higher beta cell function (HOMA-B) [10], shorter-legged children suffered more bronchitis than the long [11]. Increase in childhood length of the leg and not the length of the trunk, is related to decreased mortality due to congestive heart disease but increased risk of cancer [12] Higher risk of Prostate and testicular colorectal cancers are associated with long legs in tall people [13].

With the above studies we can put forward that the deviation of measurements from the reference values given by scholars of Ayurveda are associated with diseases and increased *Katiparinaah* and body adiposity index can be predictors of NAFLDin a very initial stage of disease. As the topographical landmarks for the measurement of *Katiparinaah* are not mentioned in the *Samhita*, so, the contemporary standard anthropometric measurement of waist circumference has been used for the estimation of *Katiparinaah*, there could be other methods also for the estimation of one *anguli* breadth like the breadth of the middle finger at the proximal inter-phalangeal joint. This may affect the result and the above values are specific to the dataset and population used for the analysis and may not be generalized to other populations without further validation. This data is from the female subjects visiting the hospital for treatment and the healthy females residing nearby villages of the hospital so it needs further validation with inclusion of the male gender, larger sample size, and populations of different geographical locations. In the present data set females of fatty liver grade III were not found. The findings of the study support the hypothesis of difference of *Katiparinaah* and BAI between NAFLD& healthy females without fatty liver and its utility in the prediction of risk of fatty liver disease of non-alcoholic origin.

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**Table 1: Distribution of Subjects in Case and Control Group**

| Variables | Control (healthy without fatty liver) | Case (NAFLD patients) |
|---|---|---|
| Number of Subjects | 87 | 66 |
| Age (mean) years | 33.55 | 38.03 |

**Table 2: Difference in *Katiparinaah* Between NAFLD and Healthy Without Fatty Liver Females**

| Variable | *Katiparinaah (in swa anguli)* | | unpaired t test | |
|---|---|---|---|---|
| | Mean | SD | t-value | *P*-value |
| Control (healthy without fatty liver) | 53.64 | 3.61 | 6.56 | <0.0001 |
| Case (NAFLD) | 58.52 | 5.65 | | |

**Table 3: ROC For The Optimum Cut-Off Point Of *Katiparinaah***

| | |
|---|---|
| Area under ROC | .768 |
| Optimum cut-off *(Katiparinaah)* | *Katiparinah* >=57.03 *anguli* |
| Sensitivity (%) | 59.1 |
| Specificity (%) | 85.1 |

**Table 4: Discrimination In Body Adiposity Index Between NAFLD And Healthy Without Fatty Liver Females**

| Variable | BAI (Body adiposity index) | | unpaired t test | |
|---|---|---|---|---|
| | Mean | SD | t-value | *P*-value |
| Control (healthy without fatty liver) | 28.5 | 2.56 | 4.12 | <0.0001 |
| Case (NAFLD) | 31.22 | 5.46 | | |

**Table 5: ROC for the Optimum Cut-Off Point Of Body Adiposity Index**

| | |
|---|---|
| Area under ROC | .645 |
| Optimum cut-off (BAI) | BAI >=32.53 |
| Sensitivity (%) | 37.9 |
| Specificity (%) | 97.9 |

**Table 6: Variation of *Katiparinaah* With Fatty Liver Grades In NAFLDPatients**

| Variable | *Katiparinaah* (*in anguli*) | | unpaired t test | |
|---|---|---|---|---|
| | Mean | SD | t-value | *P*-value |
| Grade I fatty liver | 57.28 | 5.03 | 3.33 | 0.0015 |
| Grade II fatty liver | 62.34 | 6.01 | | |

**Table 7: Variation of Body Adiposity Index with Fatty Liver Grades in NAFLD Patients**

| Variable | Body adiposity index | | unpaired t test | |
|---|---|---|---|---|
| | Mean | SD | t-value | *P*-value |
| Grade I fatty liver | 30.13 | 4.86 | 2.97 | 0.0041 |
| Grade II fatty liver | 34.57 | 6.09 | | |

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**REVIEW ARTICLE**

# Enhancing Comprehensive Biopsychosocial Assessment in Chronic Musculoskeletal Pain Management: A Multidimensional Approach

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## ABSTRACT

Chronic musculoskeletal pain (CMP) poses significant challenges in healthcare, necessitating a comprehensive understanding and management strategy. This article explores the utility of a multidimensional biopsychosocial assessment approach in CMP management. Physiotherapists, as frontline healthcare providers, play a crucial role in implementing this approach by utilizing a diverse array of subjective and objective assessment tools. The bio-psychosocial model underscores the interaction of biological, psychological, and social factors in shaping pain experiences. To address the multifaceted nature of CMP, a paradigm shift towards a bio-psychosocial model of assessment and management has gained momentum in clinical practice. Assessment tools ranging from pain intensity scales to disability assessment instruments capture various dimensions of pain perception and functional impairment. Additionally, tools targeting nervous system sensitization, fear-avoidance beliefs, psychological distress, and quality of life offer insights into contributing factors and overall well-being. The Integration of these assessment tools within a multidimensional framework enables tailored interventions to address individual patient needs effectively in health care. This comprehensive approach not only optimizes treatment outcomes but also enhances the overall quality of life for CMP patients. Continued research and clinical innovation are essential for further refining assessment techniques and treatment strategies, ultimately improving patient care and facilitating long-term recovery and functional restoration.

**Keywords:** Chronic Musculoskeletal Pain, Bio-Psychosocial Assessment, Physiotherapy, Multidimensional Approach, Pain Management.

# INTRODUCTION

Chronic musculoskeletal pain (CMP) is a complex and prevalent health concern, affecting millions of individuals worldwide and posing significant challenges for healthcare professionals. Unlike acute pain, which typically resolves with time and treatment, CMP persists over extended periods, often defying conventional therapeutic approaches. To address the multifaceted nature of CMP, a paradigm shift towards a bio-psychosocial model of assessment and management has gained momentum in clinical practice. Physiotherapists, as frontline healthcare providers, are uniquely positioned to implement this comprehensive approach, drawing upon a diverse array of assessment tools to elucidate the intricate interplay of biological, psychological, and social factors underlying CMP [1-3]. This article explores the utility of various subjective and objective assessment instruments within a multidimensional framework to enhance the management of CMP and improve patient outcomes.

**Bio-Psychosocial Assessment in Chronic Musculoskeletal Pain**

The bio-psychosocial model represents a holistic approach to understanding pain experiences, emphasizing the interaction between biological, psychological, and social factors in shaping individuals' perceptions and responses to pain stimuli. In the context of CMP, this model underscores the importance of assessing not only the physical manifestations of pain but also its cognitive, emotional, and social dimensions. Physiotherapists play a pivotal role in conducting comprehensive bio-psychosocial assessments, which serve as the foundation for targeted and personalized intervention strategies [4-6].

**Pain Assessment Tools**

Objective evaluation of pain intensity, quality, and impact on daily functioning is fundamental in guiding CMP management. A range of pain assessment tools is available to capture the multidimensional nature of pain experiences. The Numerical Pain Rating Scale (NPRS), Pain and Discomfort Module (PDM), Brief Pain Inventory (BPI), and McGill Pain Questionnaire (MPQ) are widely used instruments that provide valuable insights into various facets of pain perception and its effects on patients' lives. These tools enable physiotherapists to quantify pain severity, assess the affective and sensory components of pain, and evaluate its interference with activities of daily living [7-12].

**Disability Assessment Tools**

Assessing pain-related disability is essential for gauging the functional impact of CMP and monitoring treatment progress. Disability assessment tools such as the Shoulder Pain and Disability Scale (SPADI), Roland-Morris Disability Questionnaire (RMDQ), Oswestry Low Back Pain Disability Index, and Neck Disability Index (NDI) offer standardized measures of functional impairment across different anatomical regions. By quantifying the extent of pain-related disability, these tools assist physiotherapists in setting realistic rehabilitation goals and tracking patients' responses to intervention over time [13-18].

**Nervous System Sensitization Assessment Tools**

Central sensitization, characterized by heightened neuronal responsiveness to nociceptive stimuli, is increasingly recognized as a contributing factor in CMP. Assessment tools such as the Pressure Pain Threshold (PPT) and Central Sensitization Inventory (CSI) help identify patients with aberrant pain processing mechanisms. By quantifying pain sensitivity and central sensitization symptoms, these tools inform the selection of appropriate therapeutic interventions, such as graded exposure therapy or desensitization techniques, aimed at modulating neural excitability and reducing pain hypersensitivity [19-23].

**Fear-Avoidance Assessment Tools**

Fear-avoidance beliefs and behaviors can exacerbate pain intensity and disability in CMP patients, perpetuating a cycle of pain and functional limitations. Assessment tools such as the Fear-Avoidance Components Scale (FACS), Tampa Scale of Kinesiophobia (TSK), and Fear-Avoidance Beliefs Questionnaire (FABQ) assess the extent to which

patients' beliefs about pain influence their activity levels and functional abilities. By identifying maladaptive fear-avoidance patterns, physiotherapists can tailor interventions to address cognitive restructuring, graded exposure, and behavioral activation, thereby empowering patients to gradually resume activities and regain confidence in their physical capabilities [24-31].

**Psychological Assessment Tools**

Psychological distress, including symptoms of depression, anxiety, and pain catastrophizing, commonly coexists with CMP and can significantly impact patients' pain experiences and treatment outcomes. Assessment tools such as the Depression scale (PHQ-9), Depression Anxiety and Stress Scale (DASS), and Pain Catastrophizing Scale (PCS) provide standardized measures of psychological distress and maladaptive coping strategies. Additionally, instruments like the Pain Self-Efficacy Questionnaire (PSEQ) and Chronic Pain Coping Inventory (CPCI) assess patients' beliefs about their ability to manage pain and their repertoire of coping strategies. By addressing underlying psychological factors, physiotherapists can enhance patients' self-efficacy and resilience, fostering adaptive pain coping mechanisms and improving overall psychological well-being [32-41].

**Neurophysiology of Pain Education Tools**

Educating patients about the neurobiological mechanisms underlying pain perception is an essential component of CMP management. Assessment tools such as the Neurophysiology Pain Questionnaire (NPQ) evaluate patients' understanding of pain neurobiology and their readiness to engage in self-management strategies. By identifying gaps in patients' knowledge and misconceptions about pain, physiotherapists can tailor educational interventions to address specific learning needs and promote active participation in treatment [42].

**Quality of Life Assessment Tools**

Assessing quality of life is crucial for capturing the broader impact of CMP on patients' physical, emotional, and social well-being. Instruments such as the WHOQOL-BREF, SF-36, and EQ-5D offer comprehensive measures of health-related quality of life across various domains. By evaluating patients' perceived satisfaction with life and functional status, these tools provide valuable insights into the overall impact of CMP on their daily functioning and social interactions. Additionally, quality-of-life assessment tools can help identify areas of impairment requiring targeted interventions, such as pain management strategies, social support interventions, or vocational rehabilitation programs [43-46].

**Activity Pacing Assessment Tools**

Activity pacing is a key self-management strategy for CMP patients, enabling them to optimize their activity levels while avoiding exacerbations of pain and fatigue. Assessment tools such as the Activity Pacing Questionnaire (APQ) and Activity Pacing and Risk of Overactivity Questionnaire (APRQ) assess patients' pacing behaviors and attitudes towards activity engagement. By identifying patterns of overactivity or underactivity, physiotherapists can guide patients in developing balanced activity schedules and incorporating rest breaks to conserve energy and manage symptoms effectively [47, 48].

**Central Sensitization Syndrome Assessment Tools**

Identifying patients with central sensitization syndrome is critical for tailoring appropriate interventions to address underlying neurophysiological mechanisms. Diagnostic criteria for fibromyalgia, including the widespread pain index (WPI) and symptom severity scale (SS), help differentiate patients with central sensitization from those with primarily nociceptive pain. Additionally, tools such as the Insomnia Severity Index (ISI) and Fatigue Severity Scale (FSS) assess symptoms commonly associated with central sensitization, such as sleep disturbances and fatigue. By recognizing the broader symptomatology of central sensitization, physiotherapists can implement multimodal treatment approaches targeting pain modulation, sleep hygiene, and stress management [49-52].

**Patient Satisfaction Assessment Tools**

Evaluating patient satisfaction is essential for assessing the effectiveness of treatment interventions and identifying areas for improvement in service delivery. Instruments such as the Short Assessment of Patient Satisfaction Scale (SAPS) and Satisfaction with Life Scale (SWLS) provide standardized measures of patients' subjective experiences and overall satisfaction with care. By soliciting feedback from patients about their treatment experiences and perceived outcomes, physiotherapists can gauge the effectiveness of interventions and tailor future treatment plans to better meet patients' needs and preferences [53-55].

**Social Assessment Tools**

Understanding the social context in which pain occurs is crucial for addressing contextual factors that may influence patients' pain experiences and treatment outcomes. Social assessment tools such as the Multidimensional Scale of Perceived Social Support (MSPSS), Social Support Questionnaire (SSQ), and Work Limitations Questionnaire (WLQ) assess patients' social support networks, work productivity, and daily activities. By identifying sources of social support and potential barriers to participation in meaningful activities, physiotherapists can collaborate with patients to develop holistic treatment plans that address not only their physical symptoms but also their social and environmental context. Additionally, instruments like the Social Impact of Pain (SIP) Questionnaire and Dyadic Adjustment Scale (DAS) evaluate the broader social and interpersonal implications of CMP, helping physiotherapists recognize and address relational challenges that may arise as a result of chronic pain [56-63].

## CONCLUSION

Comprehensive biopsychosocial assessment is integral to effective CMP management, enabling physiotherapists to understand the multifaceted nature of pain experiences and tailor interventions to address individual patient needs. By integrating subjective and objective assessment tools within a multidimensional framework,physiotherapists can gain a nuanced understanding of patients' pain experiences and develop targeted treatment plans that promote optimal outcomes and improve overall quality of life. Moving forward, continued research and clinical innovation are needed to further refine assessment techniques and treatment strategies in CMP management, ultimately enhancing patient care and facilitating long-term recovery and functional restoration.

## ACKNOWLEDGEMENTS

None

### Conflicts of Interest

The authors declare no conflict of interest

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**RESEARCH ARTICLE**

# Influence of Triple Friction Pendulum Isolator Properties on Torsional Response of Building under Stochastic Ground Motions

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## ABSTRACT

Passive control systems for seismic mitigation, particularly friction pendulum bearings, have garnered significant attention in recent decades as a means to reduce earthquake demands and achieve desired performance objectives. While these bearings have proven effective in mitigating regular structures across a wide range of earthquakes, their performance and efficiency in structures with irregularities have received limited research attention. Additionally, there is a dearth of comprehensive parametric assessments examining the behavior of structures supported by friction pendulum bearings subjected to stochastic ground motions. Therefore, the purpose of this study is to evaluate the behavior of both flexible and rigid structures isolated with Triple Friction Pendulum (TFP) isolators under stochastic ground motion. The investigation is specifically assess the torsional performance of base-isolated buildings using TFP isolators with varying damping and effective time periods when subjected to stochastic ground motion. Preliminary findings indicate that the TFP bearing is effective in reducing base shear, absolute acceleration, and isolator displacement under stochastic ground motion. The superstructure flexibility is a crucial parameter for torsional response as higher torsional response has been observed in flexible superstructure than rigid superstructure. The results suggest that TFP bearings offer superior performance in mitigating seismic demands under stochastic ground motion and the torsional performance of a TFP-isolated structure improves as the isolation system's effective period increases.

**Keywords:** Seismic performance, Base isolation,Triple friction pendulum isolator, Torsional response, Stochastic ground motion, Single degree of freedom system.

# INTRODUCTION

Strong earthquakes can cause severe damage to both structural and non-structural elements of buildings, particularly in irregular structures [1-2]. Irregularities may exceeding seismic design code limits due to functional or design requirements [10-11]. Generally, integrating seismic control systems into structures becomes essential for safeguarding them against highly hazardous earthquakes. Introducing a base isolation system is recognized as one of the method of earthquake protection for structures, aiming to minimize responses such as acceleration and displacement by increasing the structure's natural time period [9,15]. Numerous studies have highlighted the significant role of base isolation systems in reducing torsional response during severe earthquakes, offering flexibility and energy dissipation capacity [6-8, 16-17].Soni et al, compared the torsional performances of double variable frequency pendulum isolated structures subjected to different ground motions [20]. Matsagar and Jangid [13-14], studied the torsional response of base isolated structure considering various isolators. A comparison of the response of the torsionally coupled base-isolated building is made with the corresponding response obtained from torsionally uncoupled base-isolated building.

In recent years, TFP bearings have gained widespread adoption as the predominant isolation system in many structures currently in operation. TFP bearings, characterized by multiple sliding surfaces with varying radii of curvature and friction, demonstrate adaptive behavior. This means that their stiffness and effective friction adjust to predictable values at manageable and controllable displacement levels. Consequently, TFP bearings can be individually optimized to address low-intensity, design-level, and maximum earthquake shaking scenarios.Fenz and Constantinou [5, 6] formulated the operational principles and force-displacement relationships of the TFP bearing from fundamental principles. They further detailed a method for modeling the TFP using an assembly of gap elements and single concave FP elements connected in series for response history analysis [5, 6].

In spite of the thorough work already done to understand the dynamic behavior the TFP bearing, there are still important aspects of the behavior of structures isolated by the TFP bearing that need further investigation especially the effect of stochastic ground motions. Hence, the response of stiff and flexible single story building isolated by the TFP Isolator is investigated under stochastic ground motions. The specific objectives of this research are (1) to study the performance of TFP isolated structure by considering super-structure eccentricity under stochastic ground motions, (2) to investigate the influence of important parameters, such as effective period and damping ratio, on the response of the TFP isolated structure. Six isolator configurations with respect to period of isolator and friction coefficient are considered. A set of 1000 stohastic ground motions are generated, having wide range variation in frequency and intensities are used.

## TRIPLE FRICTION PENDULUM ISOLATOR MATHEMATICAL MODEL

Several mathematical methods have been developed by researchers to analyze and characterize Triple Friction Pendulum (TFP) isolators. Fenz and Constantinou [4] introduced a series model where three friction pendulum elements such as friction isolator elements, gap elements, and rigid elements are connected in series. Fenz and Constantinou [5]provided a comprehensive guideline for modeling TFP isolators and offered recommendations for accurate representation. It also proposed a bi-directional model to capture the movement of each isolator part. In the mentioned study, the series model by Fenz and Constantinou [4-5] is employed, and Figure 1(a) illustrates the arrangement of the friction pendulum elements within the TFP isolator system. Figure 1(b) depicts the series model.

The characteristics of three elements in each slider ($R_{eff\ i}$,$\mu_i$ , $d_i$and $a_i$) should be chosen in accordance with Table 1 to get the tri-linear adaptive behavior of TFPBearing.

As a result, the force exerted in the x- and y-directions by the i$^{th}$ isolator element is given by,

$$\begin{Bmatrix} F_{ix} \\ F_{iy} \end{Bmatrix} = \begin{bmatrix} \frac{W}{R_{eff,i}} & 0 \\ 0 & \frac{W}{R_{eff,i}} \end{bmatrix} \begin{Bmatrix} u_{ix} \\ u_{iy} \end{Bmatrix} + \begin{bmatrix} \mu_i W_i & 0 \\ 0 & \mu_i W_i \end{bmatrix} \begin{Bmatrix} Z_{ix} \\ Z_{iy} \end{Bmatrix} + \begin{Bmatrix} F_{dix} \\ F_{diy} \end{Bmatrix}$$

$u_{ix}$ and $u_{iy}$ are the relative displacements of SFPB element $i$ in the x- and y-direction, respectively, and W is the mass supported by each isolator. Here,$\mu_i$ is the i$^{th}$ sliding surface's velocity-dependent coefficient of friction, which is given by,

$$\mu_i = \mu_{max,i} - (\mu_{max,i} - \mu_{min,i})e^{-a|\dot{x}_i|}$$

where $\mu_{max,i}$ and $\mu_{min,i}$are the coefficients of friction for sliding surfaces at maximum and minimum velocities, respectively and $a_i$ is the rate parameter of i$^{th}$ surface of the series model given by equations mentioned in Table 1 [4-5]. $a_i$ is the rate parameter of i$^{th}$surface of the TFP bearing.

$Z_{ix}$ and $Z_{iy}$ denote two dimensionless variables of i$^{th}$ element along the x- and y-directions, respectively. In the case of biaxial interaction they are governed by the following differential equations:

$$q_i \begin{Bmatrix} \dot{Z}_{ix} \\ \dot{Z}_{iy} \end{Bmatrix} = A \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \begin{Bmatrix} \dot{u}_{ix} \\ \dot{u}_{iy} \end{Bmatrix} - \begin{bmatrix} |Z_{ix}|^2[\gamma sign(\dot{u}_{ix} Z_{ix}) + \beta] & Z_{ix} Z_{iy} \left[\gamma sign(\dot{u}_{iy} Z_{iy}) + \beta\right] \\ Z_{ix} Z_{iy} \left[\gamma sign(\dot{u}_{ix} Z_{ix}) + \beta\right] & |Z_{iy}|^2[\gamma sign(\dot{u}_{iy} Z_{iy}) + \beta] \end{bmatrix} \begin{Bmatrix} Z_{ix} \\ Z_{iy} \end{Bmatrix}$$

The dimensionless numbers, $\beta$, $\gamma$, and $A$ regulate the shape of the hysteresis response, and q is the displacement quantity that represents the yield displacement of the frictional force loop. The recommended values are q = 0.25 mm, $A = 1$, $\beta = 0.9$, $\gamma = 0.1$[4-5].

According to the equation below, $F_{dix}$ and $F_{diy}$ are the forces produced in the gap element after it makes contact with the displacement restrainer.

$$F_{di} = K_G(|u_i| - d_i)sgn(u_i)H(|u_i| - d_i)$$

Where,$K_G$ is the stiffness after gap closing, which should be assigned a large value, and $H$ denotes the Heavisidefunction.

Here, a Runge-Kutta method based ODE functions (ode15s solver) that is introduced in the MATLAB programme are used to numerically resolve differential equations. Figures 2(a) and 2(b) compare the responses of single story structure introduced by Fenz and Constantinou [5] in order to test the aforementioned formulation and the subsequent outcomes.

**PROPERTIES OF THE ISOLATION SYSTEM**

In this study, TFP bearings is chosen by adjusting parameters such as the radius of the spherical surface and the coefficient of friction. The displacement capacities of all isolators remained constant. Six isolators with varying effective time periods and effective dampings are included in the current performance analysis by modifying isolator parameters. Table 2 provides the specifics of these TFP isolators.

The effective period, $T_{eff}$, and damping ratio, $\xi_{eff}$, are given by

$$T_{eff} = 2\pi\sqrt{\frac{W}{K_{eff}\ g}}$$

$$\xi_{eff} = \frac{E_{loop}}{2\pi K_{eff} D^2}$$

where $E_{loop}$ is the total energy dissipated at each cycle of the isolator displacement, $W$ is the total weight on the isolator, $K_{eff}$ is the effective linear stiffness, and D is the maximum isolator displacement under the specified level of motion

During the design process of the TFP system, it is presumed that the maximum coefficient of friction for the isolation system has been attained and that none of the individual slider displacement capacities have been reached.The TFP effective stiffness, denoted as $K_{eff}$, is derived from the geometry of the force-deformation curve, while the energy dissipated in each cycle$E_{loop}$, is determined by calculating the area enclosed by the hysteresis loop as depicted in Figure 3.

$$K_{eff} = \frac{K_1 D_y + K_2(u^* - D_y) + K_3(u^{**} - u^*) + K_4(D - u^{**})}{D} W$$

$$E_{loop} = 4(u^*)^2(K_2 - K_3)W + 4DD_y(K_2 - K_1)W + 4u^* D(K_3 - K_2)W + 4u^{**} D(K_4 - K_3)W + 4(u^{**})^2(K_3 - K_4)W + 4D_y^2(K_1 - K_2)W$$

Where

$$K_1 = \frac{1}{R_{eff2}}; K_2 = \frac{1}{R_{eff2} + R_{eff3}}; K_3 = \frac{1}{R_{eff3} + R_{eff1}}; K_4 = \frac{1}{R_{eff1} + R_{eff4}};$$

$$u^* = (\mu_1 - \mu_2)R_{eff2} + (\mu_1 - \mu_3)R_{eff3}$$

$$u^{**} = u^* + (\mu_4 - \mu_1)(R_{eff1} + R_{eff3})$$

**NUMERICAL STUDY AND RESULTS**

The current work uses a Triple Friction Pendulum Isolator (TFP) to investigate the multi-stage performance of seismically separated buildings under stochastic ground motion. In order to accomplish this, Rezaeian and Kiureghian's [18-19] stochastic ground motion model is used to develop a number of stochastic ground motions. This model accounts for the spectral and temporal non-stationarities of the motion. By altering the intensity and changing the filter's characteristics over time, non-stationarity is produced [3]. A total of 1000 stochastic ground motions are created and used for additional research. According to research [12], 1000 simulations are sufficient to accurately forecast the torsion behaviour of an isolated building. Table 3 displays the statistics of generated ground motions. These records includes a variety of intensities and frequency content.

To examine the seismic performance of TFP isolated structures, analytical simulations were conducted with varying friction coefficients and pendulum radii. Nonlinear dynamic analysis is employed to evaluate the performance of TFP isolated bearings. Parametric research is conducted using single-story buildings measuring 10m x 10m. As illustrated in Figure 4, the base-isolated building is simplified for this study as a single-story structure with masses concentrated at the upper deck and the base raft.The building's corners are supported by massless, axially inextensible columns that are fixed to a rigid foundation raft to support the upper deck. The stiff and flexible superstructures are considered, with time periods of 0.25 seconds and 1 second, respectively. A damping ratio (ξs) of 0.02 is applied. Asymmetry at the superstructure level is considered, with a ratio $\left(\frac{e_s}{d}\right)$of 0.3 throughout the study. The uncoupled torsional to lateral frequency ratio, a critical variable in a structure's asymmetric behavior, is kept constant at Ωs = Ωb = 1.

This study considers the following demand characteristics to better understand the response of the TFP isolated structure: lateral displacement at the base level, deck corner displacement, base rotation, normalized base torsion, normalized base shear, and deck corner displacement magnification. Deck corner displacement magnification is defined as the ratio of peak deck corner displacement to peak deck lateral displacement.

**Influence of Effective period of Base isolation**

The characteristics of the TFP bearing are modified by changes in the coefficient of friction and the radius of curvature of the sliding surfaces. These changes in the radius of curvature affect the isolator's time period, which consequently influences its response. To investigate the impact of the isolator's effective time period, the parameters are selected to create variations of 3 seconds, 4 seconds, and 5 seconds effective time period of isolator. For each of these time periods, the effective damping of the isolator is consistently maintained at 15%.

Figure 5 illustrates that both base displacement and deck corner displacement rise as the isolation system's effective period increases. This increase in seismic response is due to a reduction in effective stiffness, which accompanies a longer effective period. For a rigid superstructure, the increases in base displacement and deck corner displacement are 81% and 82%, respectively, while for a flexible superstructure, the increases are 77% and 67%. It is evident that changes in the effective time period have a greater impact on lateral displacement than on rotational displacement. Assuming that the translational period of an isolated system increases, the torsional period also increases, resulting in decreased base rotation as the effective period of the isolation system grows.

As expected, a structure's base shear decreases as its effective period increases. The reduction in normalized base shear is 41% for a rigid superstructure and 11% for a flexible superstructure, indicating that the impact on base shear reduction is less significant for a flexible superstructure compared to a rigid one. A similar reduction trend with increasing effective time period is observed for normalized base torsion. Figure 5 shows that the torsional performance of a TFP-isolated structure improves as the isolation system's effective period increases. The comparison reveals that the flexible superstructure is more prone to torsional response than the stiff superstructure.

**Influence of Effective damping of Base isolation**

The coefficient of friction significantly impacts the response by contributing to energy dissipation through Coulomb damping. To assess the effect of the isolator's effective damping, parameters are selected to vary the damping by 10%, 15%, and 20%. The isolator's effective time period is kept constant at 3 seconds for all damping levels. The Figure 6 shows that when effective damping increases, normalized base shear and isolator displacement decrease. The increased energy dissipation from the isolator's hysteresis behavior is what causes the reduction in isolator displacement with higher damping. The decrease in base displacement is about 20% when damping increases from $\xi_{eff}$ = 10% to 15%, but this difference narrows to 3% when damping increases from $\xi_{eff}$ = 15% to 20%. A similar trend is observed in deck corner displacement in relation to the isolator's effective damping.

When friction on the outer sliding surfaces increases, the TFP isolator helps reduce base shear and absolute acceleration. This occurs because the inner sliding surfaces, with their relatively low friction and stiffness, transmit less force to the superstructure. An increase in effective damping from 10% to 20% leads to a reduction in base shear of up to 38% for a rigid superstructure, but this effect is negligible for a flexible superstructure.Furthermore, torsional responses are more noticeable to flexible superstructures than to rigid ones. The reduction in normalized base torsion is 41% for rigid superstructures and 23% for flexible ones. It is also noticeable that the effectiveness of the TFP diminishes when the effective damping increases from 15% to 20%.

## CONCLUSIONS

The nonlinear response of a torsionally coupled system on a triple friction pendulum isolator is analyzed under stochastic ground motions. This research investigates how various significant isolator parameters affect the response of such a system. The numerical trends observed in the response of the torsionally coupled base-isolated building lead to the following conclusions.

1. The torsional response of TFP Isolated buiding is amplified by the increased flexibility of Superstructure, whilst the lateral base response and base shear is decreased. Hence, influence of flexibility of superstructure is more on torsional response of TFP Isolated building.

2. The torsional performance of a TFP-isolated structure improves as the isolation system's effective period increases.
3. The decrease in base displacement is about 20% when damping increases from $\xi_{eff}$ = 10% to 15%, but this difference narrows to 3% when damping increases from $\xi_{eff}$ = 15% to 20%. A similar trend is observed in deck corner displacement in relation to the isolator's effective damping.It is concluded that the effectiveness of the damping of TFP isolator diminishes when the effective damping increases from 15% to 20%.
4. In stiff eccentric systems, the base shear remains essentially constant for all values of effective damping and time period of isolator. While substantial effect of effective damping and time period is observed on base shear for flexible superstructure.
5. The rigid super structure assumption over-estimated the superstructure acceleration (Normalised base shear) of TFP Isolated structure.

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**Table 1 The variables of series model in terms of TFP bearings variables in a fully adaptive arrangement [6]**

| | Effective Radius of Curvature | coefficient of friction | Displacement capacity | $RateParameter$ |
|---|---|---|---|---|
| Element 1 | $R_{eff,1} = \bar{R}_{eff,2} + \bar{R}_{eff,3}$ | $\mu_1 = \bar{\mu}_2 = \bar{\mu}_3$ | $d_1 = (\bar{d}_1 + \bar{d}_2 + \bar{d}_3 + \bar{d}_4) - (d_2 + d_3)$ | $a_1 = \frac{1}{2}\ \frac{\bar{a}_2 + \bar{a}_3}{2}$ |
| Element 2 | $R_{eff,2} = \bar{R}_{eff,1} - \bar{R}_{eff,2}$ | $\mu_2 = \bar{\mu}_1$ | $d_2 = \frac{\bar{R}_{eff,1} - \bar{R}_{eff,2}}{\bar{R}_{eff,1}}\ \bar{d}_1$ | $a_2 = \frac{\bar{R}_{eff,1}}{\bar{R}_{eff,1} - \bar{R}_{eff,2}} \bar{a}_1$ |
| Element 3 | $R_{eff,3} = \bar{R}_{eff,4} - \bar{R}_{eff,3}$ | $\mu_3 = \bar{\mu}_4$ | $d_3 = \frac{\bar{R}_{eff,4} - \bar{R}_{eff,3}}{\bar{R}_{eff,4}}\ \bar{d}_4$ | $a_3 = \frac{\bar{R}_{eff,4}}{\bar{R}_{eff,4} - \bar{R}_{eff,3}} \bar{a}_4$ |

**Table 2 List of TFP bearings and their properties**

| Group | Type | $T_{eff}$ | $\xi_{eff}$ | Displacement capacity-d (m) | Effective radii-$R_{eff}$ (m) | | Friction coefficient-μ | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $R_{eff1}$=$R_{eff4}$ | $R_{eff2}$=$R_{eff3}$ | $\mu_2$=$\mu_3$ | $\mu_1$ | $\mu_4$ |
| 1 | TCFP-3-15 | 3 | 15 | 1 | 1.65 | 0.40 | 0.05 | 0.115 | 0.2 |
| | TCFP-4-15 | 4 | 15 | 1 | 3 | 0.40 | 0.02 | 0.05 | 0.1 |
| | TCFP-5-15 | 5 | 15 | 1 | 4.5 | 0.45 | 0.02 | 0.038 | 0.07 |
| 2 | TCFP-3-10 | 3 | 10 | 1 | 1.5 | 0.40 | 0.01 | 0.05 | 0.1 |
| | TCFP-3-15 | 3 | 15 | 1 | 1.65 | 0.40 | 0.05 | 0.115 | 0.2 |
| | TCFP-3-20 | 3 | 20 | 1 | 2 | 0.40 | 0.07 | 0.17 | 0.21 |

**Table 3 Statistics of generated 1000 earthquake ground motions**

| Earthquake | Maximum | Minimum | Mean | Standard deviation |
|---|---|---|---|---|
| PGA (g) | 1.5965 | 0.0717 | 0.3344 | 0.221 |
| Duration (sec) | 115.28 | 2.5 | 30.477 | 17.169 |
| Frequency *(Hz) | 106.78 | 0.03 | 30.29 | 18.52 |

* Frequency content corresponding to peak FFT amplitude of the acceleration

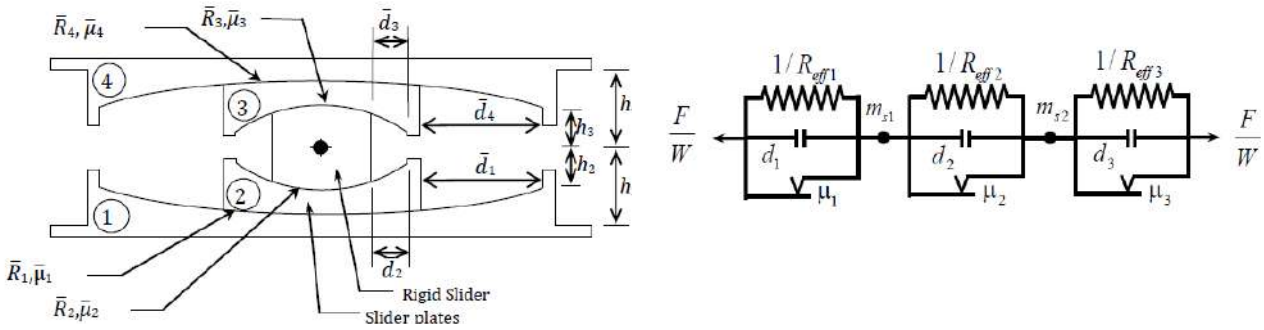
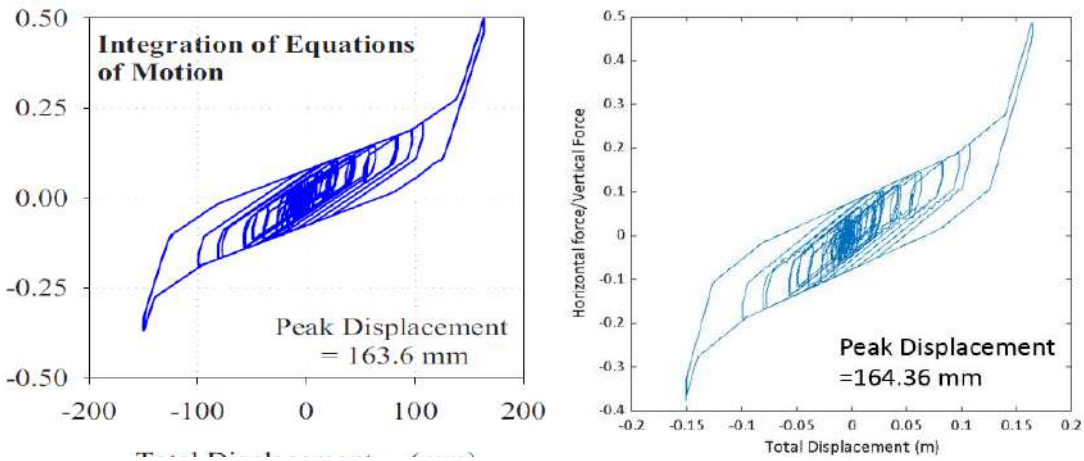



**Figure 1 Triple friction bearing (a) Schematic diagram [5] (b) Series model**




(a) (b)

Figure 2 Comparison of TFP bearing force-displacement from (a) results from Fenz and Constantinou [5]and (b) our results

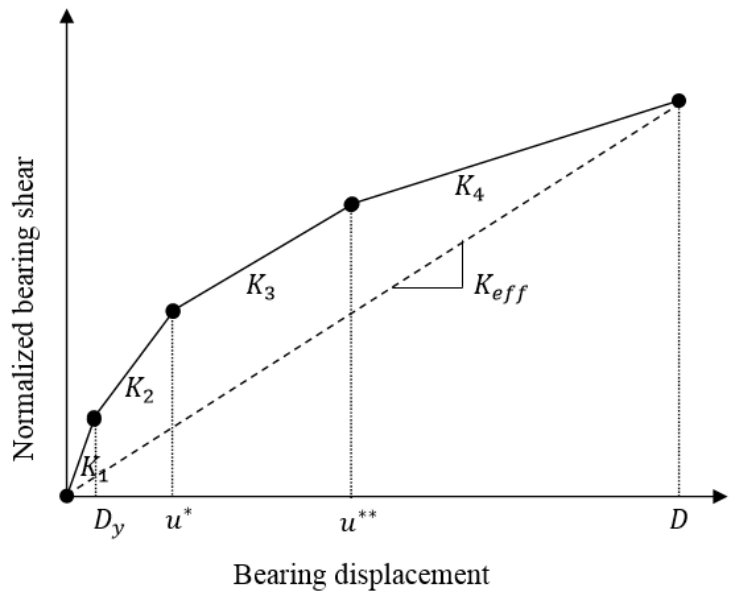
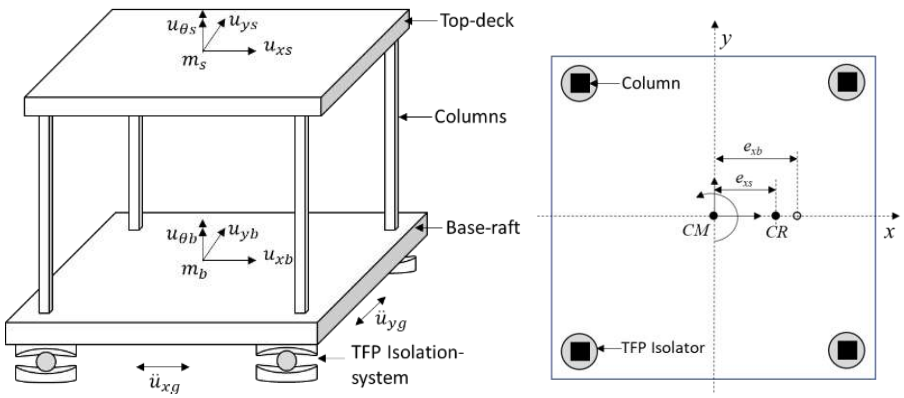



**Figure 3 Force-displacement loops of TFP Bearing**




(a) (b)

**Figure 4 (a) 3D base isolated building model (b) various eccentricities in an asymmetric base-isolated building**

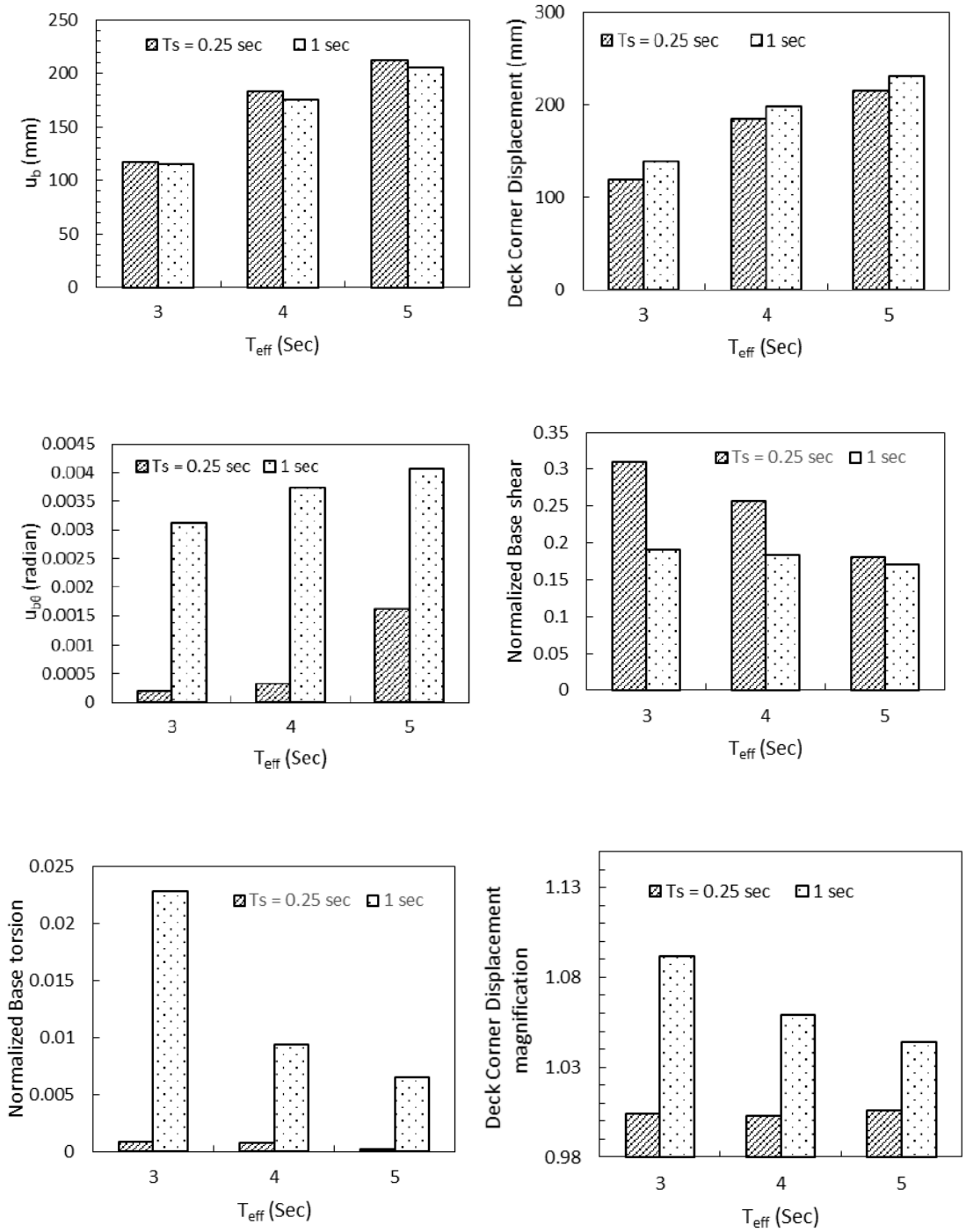



**Figure 5 Influence of effective period of isolation system for $\left(\frac{e_s}{d}\right) = 0.3$**

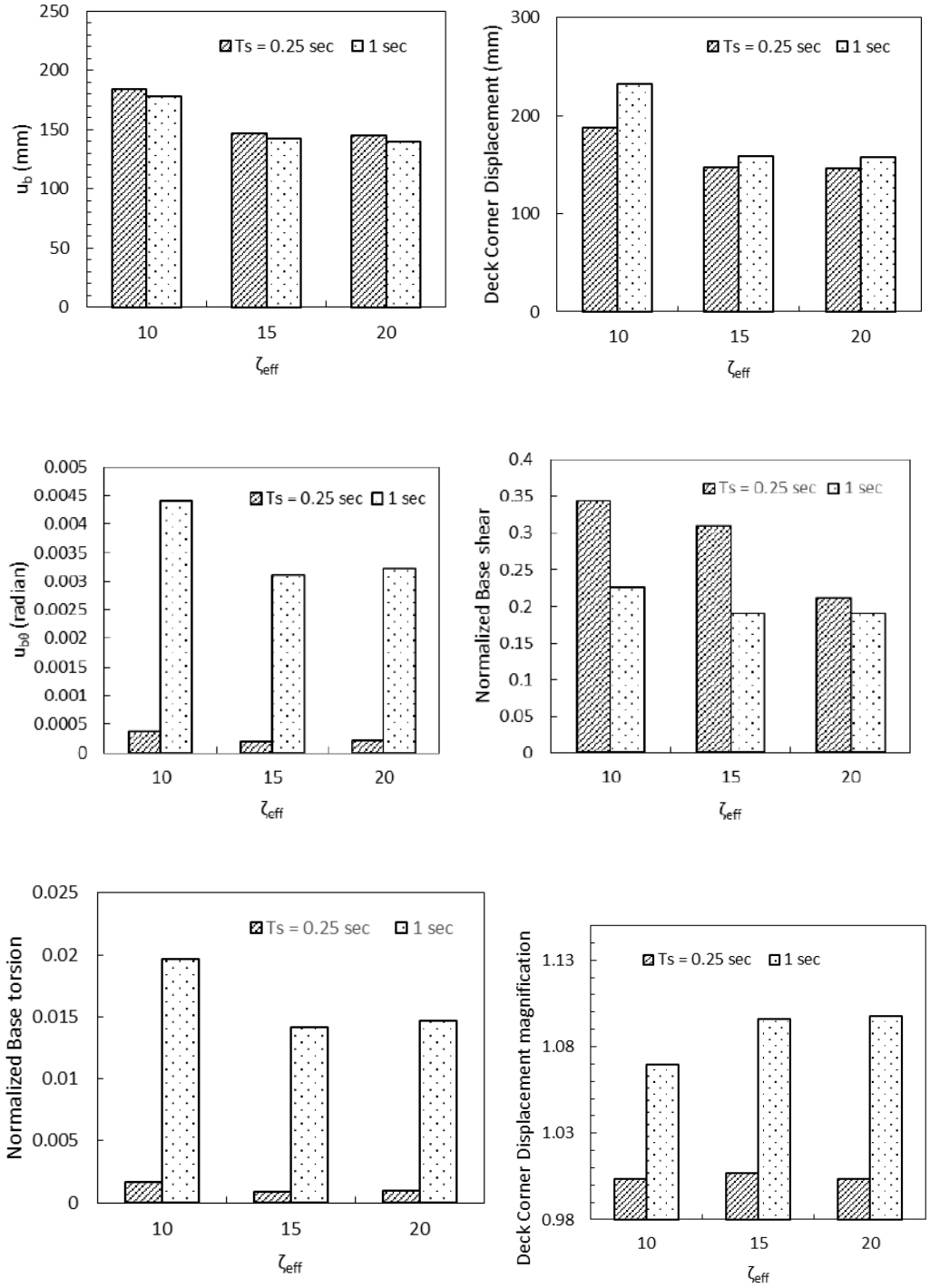



**Figure 6. Influence of effective damping of isolation system for $\left(\frac{e_s}{d}\right) = 0.3$**

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**REVIEW ARTICLE**

# Prevalence of Post Traumatic Stress Disorder among Road Traffic Accident Victims : A Systematic Review

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## ABSTRACT

The complex somatic, cognitive, affective, and behavioural effects of psychological trauma" is what posttraumatic stress disorder (PTSD) means, and it causes significant interpersonal, social, and professional dysfunction. RTAs, or road traffic accidents, are one of the biggest global issues. Traumatic injuries are very serious and leads in lifelong physical and psychological consequences. The focus on physical injuries often overshadows the lasting psychological impacts, which remain significant and intertwined with physical outcomes, necessitating comprehensive care approaches. One of the most dangerous outcomes of RTA is Post traumatic stress disorder (PTSD) as it can cause other mental diseases, this paper shows systematic review of previous studies conducted on the same subject, it gives summery of copious cross sectional along with case control study. Researcher used PRIZMA guidelines along with set inclusion and exclusion criteria to identify the research studies. Following search engines were used to retrieve the published articles on above said topic: Google scholar, PubMed, CINHAL, ProQuestand Scopus database. Research studies were analyzed and checked through NOS scale for cross sectional and case control studies for the quality assessments. The studies were taken if the score comes at medium or above. Following search strings were used "#**1**(PrevalenceOR Magnitude OR Epidemiology OR Incidence AND #**2**(PTSD OR Post-traumatic stress disorder OR Post-traumatic stress symptoms OR Stress disorder) AND #**3** (Road Traffic Accident survivors OR Road traffic accident victimsOR motor vehicle accident OR motor vehicle accidents') and #**4**(Cross sectional, OR Case control studies)". Researcher found 1447 Studies out of which 10 were selected based on the search protocol. Prevalence Variation: There is a significant variation in PTSD prevalence, ranging from 6.75% to 40.06%, influenced by country, assessment time, and specific sample characteristics. *Gender Impact:* PTSD is generally more prevalent among females across multiple studies. *Assessment Tools Consistency:* PCL-5 is the most used tool, ensuring some consistency in measurement across different settings. *Influence of Associated Factors:* A broad range of factors including personal history, accident severity, and socioeconomic impacts play crucial roles in PTSD development post-accident.

This systematic review has examined the prevalence of post-traumatic stress disorder symptoms among RTA victims. These findings underline the complexity and variability of PTSD prevalence among RTA survivors, highlighting the need for targeted interventions considering the specific associated factors identified in each study.

**Key words:** stress disorder, post-traumatic stress symptoms, RTAs, incidence

## INTRODUCTION

Annually, approximately 1.9 million people are involved in road traffic accidents. In low and middle-income countries, these incidents account for over 90% of traffic-related fatalities. In India, road transport remains the most affordable and convenient mode of travel [1]. The worst road traffic exposure stems from motorization and urbanization, making accidents and injuries a routine part of life; globally, road traffic injuries are a leading cause of death among those aged 15 to 49 years. With the increase in vehicle ownership and expanding urban areas, the frequency of such incidents continues to rise. Efforts to improve road safety and infrastructure are crucial to mitigating these risks and saving lives [2]. In 2021, there were around 1.5 lakh fatalities and 3.8 lakh injuries due to road accidents, reflecting an increase compared to the figures recorded in 2020. Out of every 100 road accidents, 37 result in fatalities(1). Victims of road traffic accidents are typically treated in emergency, orthopedic, or trauma departments for physical injuries. However, psychological issues, which may not be immediately apparent, receive the least attention. Posttraumatic stress disorder (PTSD) involves a complex range of physical, cognitive, emotional, and behavioural effects resulting from psychological trauma. These impacts infiltrate multiple aspects of a person's life, such as personal relationships, social interactions, and professional activities. PTSD leads to substantial dysfunction in these areas, disrupting daily life and impairing overall well-being(3,4). Its impact reaches beyond the individual, influencing families, communities, and broader societal structures. Therefore, addressing PTSD necessitates comprehensive strategies that recognize its intricate nature and the wide array of challenges it poses.
Numerous studies have identified varying prevalence rates of PTSD among victims of road traffic accidents (RTAs), with California notably reporting a high prevalence of 41%(4–6).

PTSD stands out as a particularly precarious outcome due to its capacity to precipitate additional mental health disorders(7). This paper presents a systematic review of previous studies focused on this subject, offering a summary of numerous cross-sectional and interventional investigations. By synthesizing existing research, it aims to illuminate the prevalence and determinants of PTSD among RTA victims(8). Through a rigorous analysis of diverse study designs and methodologies, it provides insights into the multifaceted nature of PTSD following RTAs. The findings contribute to a deeper understanding of the psychological impact of RTAs and emphasize the urgent need for effective interventions to address the mental health challenges faced by survivors. Such efforts are crucial for enhancing the overall well-being and quality of life of individuals affected by traumatic accidents.

**Rationale for the study**

Literature that could validate the above statement along with prevalence of post-traumatic stress disorder. Many researchers investigated the prevalence of PTSD among different population but there are only few on road traffic accident victims, which indicates research gap in precise investigation on prevalence of PTSD among road traffic accident victims. The collected evidence from literature review showed that there is need for evidence-based intervention to treat PTSD, the researcher wants to conduct a study on effectiveness of mental health first aid kit on PTSD therefore, there is a requirement to know the magnitude and trend of prevalence so that it helps to plan intervention accordingly.

**Research question, Aim and objective**

Research question is what is the prevalence of post-traumatic stress disorder among road traffic accident victims? The primary aim of the research is to conduct a systematic review to identify the prevalence of PTSD among road traffic accident victims. Objectives of the study was to understand the characteristics of the study participants, instrument used used to assess the PTSD, time when PTSD is assessed and to identify the prevalence rate of PTSD.

## MATERIALS AND METHODS

This systematic review was conducted adhering to PRISMA(9).We conducted a comprehensive systematic review published up to 2024 to identify the prevalence of PTSD among road traffic accident victims.

**Study Selection**

The inclusion criteria were oriented along with the PICOS elements. Regarding the population (P), we included study conducted in any country that involves road traffic accident victims. We do not exclude any age group and we also had no restriction on gender or type of accident. In terms of intervention or exposure (I) we included victims exposed to any kind of road traffic accident, we do not exclude victims based on vehicle or body parts injured. In terms of comparison (C) we didn't compare any variables in the study. In terms of outcome (O), Eligible studies had to include outcome related to prevalence rate of PTSD. Regarding study design (S), we included cross sectional and case control study studies (Cross sectional and cross-sectional survey), we also included studies having prevalence of PTSD along with assessment of any other mental health issues. The reason for including cross sectional studies is they are best way to determine the prevalence and can study the association of multiple exposure and outcomes.

**Inclusion and exclusion criteria:**

**Studies were included if they met the following criteria:**

The articles of peer-reviewed journal as they have validated knowledge and result, published in the English language, published from the last ten years to utilise current and reliable result, based on the cross-sectional study to gather relevant evidence, articles focusing on prevalence of PTSD reference to RTA and studies on victims like pedestrian, driver, passenger, cyclist, bikers and event witnesses.

**The exclusion criteria are:** The articles with half text, not fully accessible, articles in a foreign language, articles older than 10 years, qualitative studies, conference papers, posters and dissertations are excluded due to quality concern and studies on PTSD other than road traffic accident victims.

**Data sources and searches**

The search was undertaken in the high scholarly electronic databases like, Google scholar, PubMed, CINHALS, Scopus and ProQuest for relevant peer-reviewed scientific articles. Boolean operators such as AND, OR and search limit was used in the search strategy. The reason for using multiple databases is to have a high quality, peer reviewed literature, to increase the reliability and validity of the review and to eliminate the reporting bias and showing credibility. A broad search string was used that included a combination of appropriate keywords such as:

**Database:** Google Scholar, PubMed, CINAHL, Scopus, and ProQuest are essential databases used to search for scholarly studies

**Search Strings:** #1(Prevalence OR Magnitude OR Epidemiology OR Incidence AND #2(PTSD OR Post-traumatic stress disorder OR Post-traumatic stress symptoms OR Stress disorder) AND #3 (Road Traffic Accident survivors OR Road traffic accident victims OR motor vehicle accident OR motor vehicle accidents') and #4(Cross sectional, OR Case control studies)" used for advance search in database.

**Quality assessment:** The Newcastle-Ottawa scale (NOS) instrument was used to assess the quality of studies. In cross section studies we use an adaptation to specific criteria for sampling, non-response rate and statistical analysis employed. The NOS instrument includes items divided in three areas, selection, comparability and outcome. Maximum score is 9, 4 points for selection, 2 points for comparability and 3 points for outcomes. (See table no.1).

**Data collection**

The researcher reviewed the articles' titles and abstracts using the pre decided keywords to determine their appropriateness for this systematic review. Eligible articles were evaluated and scored using the Newcastle-Ottawa scale (NOS). If an article scored > 7, 8 or 9 on the NOS scale and met all other inclusion criteria, it was included in the review.

**Data extraction**

From each of the study, information based on different study parameters was extracted that help in retrieving the reliable result (see table no. 2). The parameters included study characteristic like author name and year of publication, country, study design, study participants, sample size, instrument used to assess PTSD, Time when PTSD is assessed and prevalence rate with associated factors.

## RESULT

**Study selection:**The initial database search yielded a total of 1447 records from various sources, including PubMed (n=481), Google Scholar (n=194), CINHAL (n=3), ProQuest (399) and Scopus (n=370). After removing duplicates, 1028 unique records remained for further analysis. From these, 913 records were excluded after abstract review, 115 full-text articles were assessed for eligibility, leading to the inclusion of 10 articles for the systematic review. This rigorous process ensured a comprehensive and methodical approach to identifying relevant literature for the study. The table no 2 provides a comparative overview of various studies examining the prevalence of PTSD among survivors of road traffic accidents (RTA) across different countries, study designs, participant types, sample sizes, assessment tools, time frames, and associated factors.

**Country and Study Design:** The studies span a range of countries, including Ethiopia, Benin, India, Iran, the USA, and Israel. Most studies employ a cross-sectional design, except for Yimer et al. (2023), which uses a case-control approach.

**Participants and Sample Size:** Participants across studies vary, including survivors of RTAs, drivers, pedestrians, motorcyclists, and passengers. Sample sizes range from 250 (17) to 865 (11)Age and Gender: *Median/Mean Age:* Ages vary, with median or mean ages typically in the 30s. For instance, Yimer et al. (2023)(10) report a median age of 38, whereas Donatien Daddahet al. (2022)(11) report a mean age of 38.04.*Gender Distribution:* Gender distribution varies, with some studies reporting higher male participation (e.g., Yohannes et al., 2018: 63.6% male)(16), while others have a higher female prevalence in PTSD rates (e.g., Yimer et al., 2023: 58.52% female among cases)(10). Instruments Used: The studies predominantly use the PTSD Checklist for DSM-5 (PCL-5) for assessment, though some utilize the DSM-5, Trauma Screening Questionnaire (TSQ), and Clinician-Administered PTSD Scale (CAPS). *Time of PTSD Assessment:* The timing of PTSD assessment post-accident varies: Short-term(1 month to 3 months): Most studies assess PTSD within this period(10,12,16). Long-term (up to 12 months): Donatien Daddahet al. (2022)(11) and Arora et al. (2017) (17)extend the assessment to 12 months post-accident.

**Prevalence Rates:***Highest Rates:* Hamid Soori et al. (2021)(13)report the highest prevalence at 40.06%. *Lowest Rates:* R. Naim et al. (2014) (19)and Hruska et al. (2014)(18) report the lowest prevalence at 6.75% and 10%, respectively.

**Associated Factors:** Common factors associated with increased PTSD prevalence include*Gender:* Higher prevalence in females(10,11). *Personal and Psychological Factors:* History of psychiatric issues, depression, and presence of

comorbidities(10,16). *Accident-related Factors:* Presence of fractures, witnessing death, hospitalization, and severe risk behaviors (13,15). *Social and Economic Factors:* Impact on income, social support, and return to work (11,16).

## DISCUSSION

A total of 10 studies, including 4,272 samples, were analysed to assess the prevalence of PTSD among road traffic accident victims. The primary objective of the research was to evaluate the prevalence of PTSD in this group. The studies collectively aimed to provide a comprehensive understanding of PTSD prevalence among these victims.Individuals who suffer from traumatic injuries often face a diminished quality of life and significant mental health challenges. They tend to have high rates of recurring injuries, dissatisfaction with pain management, and low levels of engagement in post-injury care(20).

**Prevalence of the PTSD among RTA victims**

PTSD commonly observed in RTA victims. Current study, Prevalence rates range from 40.06% at the highest to 6.75% and 10% at the lowest reported levels. The differences in prevalence may stem from geographical factors and the lack of psychological care provided in emergency departments. This study contributes to the current data by emphasizing the essential need for psychological care among motor vehicle accident (MVA) survivors attending the orthopaedic and trauma clinic at Kenyatta National Hospital, Nairobi(21). Furthermore, the prevalence of PTSD is influenced by various factors, with age and experiences of significant loss playing crucial roles. Older individuals, who may have experienced more traumatic events over their lifetimes, are more susceptible to developing PTSD, especially following the death of a close family member or friend. This finding supports the article, showing a 46.5% incidence of PTSD among RTA survivors, linked to witnessing death during the accident, severe family relationship impacts, and previous psychiatric illness(22). These factors underscore the multifaceted nature of PTSD development in trauma survivors These experiences can amplify feelings of grief and trigger PTSD symptoms. Conversely, gender and role in motor vehicle accidents (MVA) can contribute to lower PTSD prevalence rates Females often report higher levels of PTSD due to greater susceptibility to the emotional impact of trauma, whereas individuals in less directly involved roles in MVAs, such as witnesses or bystanders, may experience lower levels of PTSD due to reduced proximity to the traumatic event and potentially less severe emotional impact. The results align with earlier research: men tend to experience more traumatic events, while women exhibit more severe psychiatric disorders following exposure(23). This finding is supported by the gender-specific role of peritraumatic dissociation study, which corroborates the gender differences in trauma exposure and its psychological impacts.These factors highlight the complex interplay between personal circumstances and PTSD prevalence.

**Advancing PTSD Research among Drivers and RTA Survivors**

Future research on PTSD among drivers and road traffic accident (RTA) survivors can significantly benefit from the strengths identified in recent studies. Utilizing standardized instruments like the PTSD Checklist for DSM-V (PCL-5) ensures consistent and reliable measurement of PTSD symptoms, facilitating comparability across studies. Adopting well-defined catchment areas and sampling from multiple hospitals enhances the representativeness of samples, crucial for generalizing findings to broader populations. Moreover, employing large and diverse samples improves statistical power and allows for subgroup analyses, identifying nuanced risk factors and protective factors. The involvement of trained health professionals and supervision by medical epidemiologists ensures rigorous data collection and adherence to high research standards. By leveraging these strengths—standardized assessment tools, representative sampling strategies, large sample sizes, and expert involvement—future research can deepen our understanding of PTSD dynamics in this population, informing more targeted interventions and support initiatives.

**Strength and weakness of reviewed articles**

All the studies reviewed share several common strengths and weaknesses, highlighting both their robust methodologies and limitations. First, the strength of using standardized instruments for measuring PTSD, such as the PTSD checklist for DSM-V (PCL-5)/PTSD checklist-Specific version (PCL-S) or similar tools, is a notable consistency

across the studies(10,11,14–17).These standardized tools ensure a reliable assessment of PTSD symptoms. Moreover, several studies benefitted from sampling participants from well-defined catchment areas or multiple hospitals, which helps in obtaining a more representative sample of the target population(11,14–16). However, these studies also share common limitations. The cross-sectional design prevalent in all studies(10,11,14–17). Limits the ability to establish causality between PTSD and various factors. The focus on drivers or subjects with recent road traffic accidents (RTAs) makes it challenging to generalize findings to those without RTAs or with different trauma histories. Additionally, the non-random sampling techniques and smaller sample sizes employed in some studies(10,14,17). further limit the generalizability of the results. Lastly, potential recall and social desirability biases may have influenced the participants' responses, affecting the reliability of the findings(11,17).

**Limitations:**
The main limitation of this study included the database from Google scholar, PubMed, ProQuest, CINHAL and Scopus database. Authors were limited access to paid articles, incomplete text and studies in other languages. Type of studies were limited to the purpose of the systematic review.

## CONCLUSION

This systematic review has examined the prevalence of post-traumatic stress disorder symptoms among RTA victims. Understanding the multifaceted dynamics influencing PTSD among RTA survivors underscores the need for comprehensive support systems and tailored interventions. Strengthening social networks, promoting mental health awareness, and addressing gender-specific needs can enhance resilience and mitigate the long-term impact of trauma on survivors' well-being(1,24,25).

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**Table:1 Newcastle -Ottawa (NOS) Quality assessment scale is used for cross section or case control studies**

| Sr. No. | Author and publication year details | Selection | | | | Comparability | Outcome | | Total scoring |
|---|---|---|---|---|---|---|---|---|---|
| | | Representativeness of the sample | justified sample size | There was satisfactory response rate | Ascertainment of the exposure | potential confounders were checked | Outcome assessment | Statistical analysis | |
| 1 | Yimer et al., | * | * | * | ** | ** | ** | * | **9** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (Y:2023)(10) | | | | | | | | |
| 2 | Donatien Daddahet.al., (Y:2022)(11) | * | * | * | ** | - | ** | * | **08** |
| 3 | Ratnani, D (Y:2022)(12) | * | * | * | ** | - | * | * | **07** |
| 4 | Hamid Soori, et al., (Y:2021)(13) | * | - | * | ** | - | ** | * | **07** |
| 5 | Bedaso et al, et al., (Y: 2020) (14) | * | * | * | ** | ** | ** | * | **09** |
| 6 | Alenkoet al.,(Y: 2019) (15) | * | * | * | ** | * | ** | * | **09** |
| 7 | Yohannes e t al., (Y:2018)(16) | * | * | * | ** | * | ** | * | **09** |
| 8 | Arora, et al., (Y:2017) (17) | * | * | * | ** | - | ** | * | **08** |
| 9 | Hruska et al., (Y:2014)(18) | * | - | * | ** | * | ** | * | **08** |
| 10 | R. Naim et al., (Y:2014)(19) | * | - | * | ** | * | * | * | **07** |

**Table 2 Summary of Reviewed Articles.**

| Sr. No. | Author and publication year details | Country | Study design | Participants | Sample size | Instrument used | Time when PTSD is assessed | Prevalence rate |
|---|---|---|---|---|---|---|---|---|
| 1 | Yimer et al., (Y:2023) (10) | Ethiopia | case–control study | RTA Survivors | 419 (135 cases) samples. Median age 38 Male: 56(41.8%), Female: 79 (58.52%) | PCL-S | After 1month | 33.17% Associated factors: Gender ( more in females), education, presence of personal psychiatric history, presence of |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | fracture, witness of death, presence of comorbidity and social support |
| 2 | Donatien Daddahet.al., (Y:2022)(11) | Benin (West Africa) | Cross-sectional study | pedestrian, motorcyclist, Drivers & other | 865 Sample Mean age: 38.04 Male: 73.98%, Female:26.2% . | PCL-S | After 12 months | 26.43% Associated factors: Gender ( More in female), Hospitalization, negative impact of accident on income and no return to work |
| 3 | Ratnani, D (Y:2022)(12) | India | Cross-sectional study | RTA / MVA Survivors | 300 samples | DSM-5 | After 1 month | 22.6% Associated factors: Poor roads, violation of traffic rules, gender ( more in female) |
| 4 | Hamid Soori, et al., (Y:2021)(13) | Iran | Cross-sectional study | RTA Survivors | 350 sample | PCL-5 | After 1 week to 2 months | 40.06% Associated factors:Age, deathof close family or friend |
| 5 | Bedaso et al, et al., (Y: 2020)(14) | Ethiopia | Cross-sectional study | Driver & assistant passenger, Pedestrian | 423 sample Median age:30 | PCL-S | After 1 month to 3 months | 15.4% Male: 15.8%, female: 14.2%. Associated factors: History of previous accident, Time since accident and depression |
| 6 | Alenkoet al., (Y: 2019) (15) | Ethiopia | Cross-sectional study | Drivers | 402 sample Mean age: 32.11 | TSQ | After 1 month | 12.6% Associated factors: Near miss RTC's, depression and severe risk |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | cannabis use |
| 7 | Yohannes et al ., (Y:2018) (16) | Ethiopia | Cross-sectional study | RTA Survivors | 492 Sample Mean age: 30.12 Male (63.6%) Female(36.4 %) | PCL-5 | After 1 month | 22.8% Female: 32.4%, Male: 17.25% Associated factors: Gender (Higher rate in female), Social support, duration since accident and depression. |
| 8 | Arora, et al., (Y:2017)(17) | India | Cross-sectional study | Driver, Pillion driver, Passenger, Pedestrian | 250 sample Male: 77.2%, Female:22.8% | PCL-S | After 1 month to 12 month | 32.4% Associated factors: Age more than 45 years, sustained fracture, sued substance while driving, and blamed self for accident, perceived threat from accident. |
| 9 | Hruska et al., (Y:2014)(18) | USA | Cross-sectional study | MVA Victims | 356 Sample Male: 211(59.3%), Female:145 (40.7%) Mean age 38.66 | CAPS, TSS | After 6 weeks | 10% |
| 10 | R. Naim et al., (Y:2014)(19) | Israel | Cross-sectional study | Driver, Motorcyclists, Bicycle riders, Pedestrians and passengers | 415 Sample Mean age:35.02 | CAPS | After 3 month | 6.75% Male:1.08% , Female: 10.8% Associated factors:Gender , Role in the MVA |

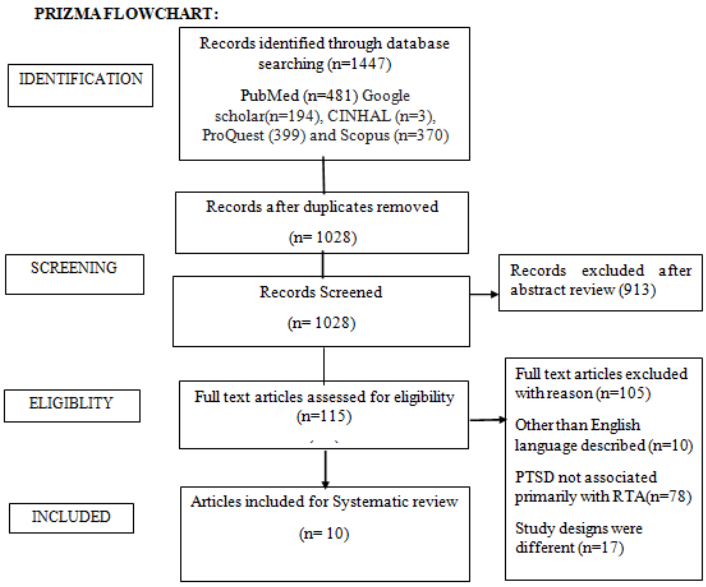


**Fig.1 Prizma Flowchart**

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**REVIEW ARTICLE**

# Ethnomedicinal, Nutritional and Bio-Ceutical Benefits of *Carica papaya* : A Comprehensive Review

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**ABSTRACT**

For many centuries, humans have been dependent ontraditional medicine, due to its high safety, affordability, and acceptability. With the increase in the demand for functional food in the Indian pharmaceutical and nutraceutical market, plant materials with high bio ceutical properties are much in demand. One such plant is *Carica papaya* of family Caricaceae better known as papaya. As per multiple reports, different parts of papaya have been traditionally used to control, cure, and rehabilitate against different diseases. The reports suggest that papaya contains different phytochemical analogs of carpaine, kaempferol, and quercetin along with various enzymes and other proteins which are responsible for its, antioxidant, anti-cancer, anti-inflammatory, immunomodulatory activities and wound healing properties among many others along with improvement in skin and gut health. All these properties directly point towards a highly potential bio ceutical plant material in the form of papaya. With potent clinical studies, papaya can become a trailblazer in the field of pharmaceutical and nutraceutical industries.

**Keywords:** *C. papaya*, bio ceutical properties, phytochemicals, anti-microbial, anti-cancer.

## INTRODUCTION

Herb and herb-based products have been used in traditional medicine for several centuries to treat various diseases. Multiple studies have shown that around three-quarters of the population of the world depends on herb-based medicine for their primary health care. There are various advantages of the natural product over the much-hyped synthetic products like affordability, ease of availability, lower toxicity, lower adverse effects as well as low chances

of multi-drug resistances. In recent years, the dependence on natural products mainly from plants and herbs has majorly increased due to the bio-ceutical revolution, where the usage of plant products as functional foods and neutraceuticals has played a key role. Here plants and plant products can neither be categorized as food nor as pharmaceuticals, but more as supplements to control, prevent, ameliorate as well as rehabilitate certain critical diseases. Many plants like garlic, ashwagandha, spirulina, chicory, fenugreek, etc. have found market share worth millions of dollars around the world due to the bio-ceutical revolution. In this study a plant very commonly seenaround India, that is *Carica papaya* will be thoroughly emphasized through different reports of its curative properties. [1].

*Carica papaya*, the well-known papaya, is a plant that belongs to the Caricaceae family. It contains a variety of chemicals and biomolecules, including papain, which has important uses in industry and medicine. Originating in Central America and Mexico, papaya is an evergreen plant that grows in tropical climates[2,3]. There is little evidence to suggest that it originated in the northwest of South America[4] and was introduced to India in the sixteenth century.

*C. papaya*, also referred to as papaya, has anti-inflammatory effects, particularly in the leaves and seeds [5]. According to research, papaya leaves contain chemicals that may aid the immune system manage its inflammatory response [5]. Research has also shown that papaya leaf extracts can reduce inflammation in a variety of cells, including those involved in wound healing [6,7]. Papaya seeds appear to have anti-inflammatory properties, presumably by lowering oxidative stress and inflammation in the liver[8]. In this study, comprehensive details regarding the ethnomedicinal, bioceutical, and nutritional benefits of papaya have been discussed.

## TAXONOMY, MORPHOLOGY, AND DISTRIBUTION OF *C. papaya*:

### Botanical Classification

The domain of *C. papaya* comes under the flowering plant, while the kingdom and sub-kingdom of the plant are 'Plantae' and 'Tracheobionta' respectively. Class and sub-class of*C. papaya*is 'Magnoliopsida' and 'Dilleniidae'. Phylum, order, family, and genus of Carica papaya are 'Steptophyta', 'Brassicales', 'Caricaceae', and 'Carica'. Thus the botanical name of *Carica papaya* is as following *Carica papaya* Linn. [9]

### Morphology of *C. papaya* tree

The papaya plant is often a single, hollow, erect-stemmed, semi-woody perennial tree that grows to a height of around 20 to 30 feet. The stem is 8 inches in diameter, light green to tan brown, and bears leaf scars. It has a cluster of huge, palmately-lobed leaves with 25–100 cm long petioles. Depending on the sort of blossom they produce, papaya plants can be categorized as male, female, or hermaphrodite. Some plants are known as monoecious because they may produce both male and female blooms[10].

### Distribution of *C. papaya*

The specific region of origin for papaya is unknown. It is thought to be native to Tropical America, possibly in Southern Mexico and adjacent Central America. Today, successful commercial production is focused largely in Hawaii, Tropical Africa, the Philippines, India, Sri Lanka, Malaysia, and Australia; South Africa and Latin America produce on a smaller scale [2,3,4].

### Nutritional Content of *C. papaya*

In today's world, conventionally synthetically generated medications are the primary treatment approaches. However, due to their unfavorable side effects in both long and short-term consumption of drugs, alternative pharmaceutical systems are becoming increasingly popular due to their lower adverse effects and higher consumer compliance. C. papaya, sometimes known as papaya, is an alternative natural medicine source. The pulp, leaves, and seeds contain high levels of vitamins, bioactive compounds, and lipids that prevent hypercholesterolemia, protect

against thrombogenesis and oxidative stress, and lower inflammatory markers and anti-platelet aggregation—all of which can be caused by obesity [11,12].

**Vitamins and Minerals**

Papaya is a very common fruit with a low price and excellent nutritional content. It has fewer calories and more vitamins, macronutrients, and other minerals, making it ideal for individuals who are in danger of gaining weight. There are several macronutrients found in *C. papaya* fruit [13]. Carbohydrates are the most prevalent macronutrient in *C. papaya* (7.2 gm per 100 mg), but proteins, lipids, and fiber are also present. Papaya contains a large quantity of energy, with 32 kcal per 100 mg. The most abundant minerals in *C. papaya* fruit are sodium, potassium, calcium, magnesium, phosphorus, and iron [14]. Various vitamins and their complexes are present in *C. papaya* fruit [15]. Papaya is a Vitamin A-rich fruit. Also Vitamin B complexes like B1,B2,B3,B6, B9, and Vitamin C are also present.

**Enzymes**

Papaya consists of many enzymes that serve various purposes. Enzymes typically induce proteolytic activity. However, enzymes can help the digestion of a variety of macronutrients. Papaya enzymes have various roles such as immunomodulation and anti-inflammatory effects[16,17,18]. Papaya proteases have anti-inflammatory qualities that aid in relieving pain and suffering from arthritis, edema, and osteoporosis. Enzymes in *C. papaya* are broadly distributed throughout the plant. It contains cysteine endopeptidases such as glycyl endopeptidase, cysteine proteinases, serine proteinase inhibitors, glutaminyl cyclase caricain, class II chitinase, papain, and chymopapain. Papain is a non-specific thiol protease that acts similarly to pepsin in gastric juice, making it a good digestive aid and pepsin dilapidation agent. [19]. Other active compounds of *C. papaya* are lipase, a hydrolase, which is tightly bonded to the water-insoluble fraction of crude papain and is thus considered a "naturally immobilized" biocatalyst [20]. Pectinase, found in papaya fruit, is involved in the breakdown of pectin, a carbohydrate found in plant cell walls. The seeds of papaya include lipase and amylase enzymes, which help to break down lipids and starch. Cellulase, which is found in plant leaves, helps to break down cellulose into simple sugar. Younger plants have more of these enzymes than older plants.

**Bioactive Compounds of *C. papaya***

Bioactive compounds are naturally occurring chemical components found in various plant parts that provide medical benefits to many animals. Chemical investigations conducted in several research revealed that papaya leaves contain several phytochemicals that have been shown to benefit health. These phytochemicals contain considerable levels of alkaloids, saponins, glycosides, flavonoids, phenolic compounds, enzymes, amino acids, lipids, carbohydrates, vitamins, and minerals [21]. Papaya leaves have been used medicinally in numerous Asian countries for centuries to treat a variety of ailments. Because of the presence of the aforementioned critical operating components, they are used to treat a wide range of illnesses, including diabetes, ulcers, hypertension, dengue, menstruation problems, dermatitis, sinusitis, weakness, and others [22]. Papaya leaves contain seven flavonoids: quercetin, quercetin3-(2G-rhamnosylrutinoside), quercetin 3-rutinoside, kaempferol 3-rutinoside, kaempferol 3-(2Grhamnosylrutinoside), and myricetin 3-rhamnoside. The leaves include phenolic compounds such as caffeine, protocatechuic acid, quercetin, 5,7-dimethyl coumarin, p-coumaric acid, and chlorogenic acid [23]. These phenolic chemicals, in particular, contribute to anti-allergic, antiviral, anti-inflammatory, and anti-cancer capabilities, as well as anti-dengue activity. The key phytochemicals found in papaya plant leaves are papain, cystatin, chymopapain, tocopherol, phenolic acids, cyanogenic glucosides, glucosinolates, and vitamin C, which can improve the general anti-oxidant qualities of the blood.Papaya leaves have been utilized as antispasmodic, analgesic, and antibacterial because they contain numerous alkaloids such as carpaine, pseudocarpaine, dehydro-carpaine, and phenolic compounds. Carpaine, dehydrocarpaine I, and dehydrocarpaine II are the principal bioactive components found in papaya leaves. Because of the presence of carpeting, these herbal leaves are used in Ayurvedic formulations to treat a variety of physical ailments and viral fevers such as dengue and chikungunya. Carpaine has also been shown to have strong anticancer and antihelminthic effects [24]. Its content has been reported to be maximum in mature papaya leaves. Researchers recently found that malaria can be efficiently cured with papaya leaves. This beneficial impact is attributed to the presence of alkaloids in the leaves, which contain quinine, an antimalarial drug [25,26]. Papaya leaves contain a high concentration of

biological enzymes, specifically papain and chymopapain. The concentration of papain in papaya leaf extract present in significant amounts [15] and due to which very powerful digestive action even higher than pepsin is seen. Mainly alkaloids, saponins, glycosides, phenolic compounds, and flavonoids are responsible for the anti-inflammatory and anti-cancerous properties of papaya leaves [27].

**Antioxidant**

Due to concerns about the toxicity of synthetic antioxidants, plant-derived compounds are often sold as natural antioxidants as an alternative. Oxidative chemicals found in many plants may have antibacterial, antiviral, and anticancer effects. They also have an impact on other health benefits [23]. Several components in the anti-oxidant family, such as carotenoids, vitamin C, vitamin E, and phenolic compounds, exert anti-oxidant action through interaction. As a result, it is difficult to determine C. papaya's total antioxidant activity solely on individual active components [28]. The total antioxidant level in C. papaya fruit varies significantly as it ripens. Antioxidant levels gradually rise as the fruit matures. Free radicals generate oxidative damage that has a significant impact on many chronic diseases, and antioxidants can help our health by reducing the production of free radicals. New sources of natural antioxidants that are both safe and economically viable are currently being studied [29]. Researchers created silver nanoparticles from C. papaya peel extract (CPPE) and tested their antioxidative capabilities to confirm their efficiency. It was discovered that the concentration-dependent activity of AgNPs was 56% for synthesized AgNPs and 38% for commercially available CPPE [30]. In the whole plant, the fruit and the seeds contribute to the anti-oxidant property the most. Various tests to determine the anti-oxidant properties e.g. TPC( Total phenol content), TFC( Total flavonoid content), FRAP( Ferric reducing antioxidant power ), Radical scavenging activity of various extracts of solvents like n-butanol, Petroleum Ether, Methanol, Ethyl Acetate, etc. Out of the extracts, Ethyl acetate and n-butanol fractions hold the strongest anti-oxidant activity [31]. The antioxidant potential of papaya peel may contribute to the production of functional foods and nutraceuticals shortly [32]. To demonstrate antioxidant activity, researchers treated papaya peel residues with ethanol and dried them in a microwave oven to produce dietary fiber concentrations (DFCs). The samples' chromatographic analysis revealed the presence of carotenoids, phenolics, ascorbic acid, proteocatechuic acid, manghaslin, quercetin 3-O-rutinoside, caffeoyl hexoside, ferulic acid, lutein, zeaxanthin, and beta-carotene. Digestibility studies revealed that the percentage of indigestible fiber included antioxidants. Salla et al. studied the in vitro antioxidant activity of papaya peel extracts as well as their effects on endogenous glutathione, superoxide dismutase, catalase, cyclo-oxygenase-2 (COX-2), cyclo-oxygenase-3, and DNA fragmentation in HepG2 cells[33].

**Antimicrobial effects**

Papaya seed exhibits antibacterial action directed against the trophozoites of *Trichomonas vaginalis*. According to the paper, papaya seed should be used carefully to prevent toxicity in urinogenital disorders like trichomoniasis.Papaya seed is used in urinogenital disorders like trichomoniasis with care to avoid toxicity [33]. With the agar cup plate method, it was demonstrated that the papaya seed and pulp had bacteriostatic properties against a range of enteropathogens, including *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*. [34].It has been demonstrated that the papaya fruit, seeds, leaves, and peels of *C. papaya*have antibacterial properties against the possibly harmful microbe.. the seed and pulp showed bacteriostatic properties against several enteropathogens such as *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus sp.C. papaya* could find usefulness in the production of drugs against organisms causing urinary tract infections [35]

***Antibacterial activity***

The results of an investigation indicate the antibacterial properties of papaya fruiting body extracts. It is most likely for this reason that some villagers treat wounds with paw-paw. Although it has no impact, the extract is bacteriostatic and bactericidal. This partially defies Vieria's results [36]**.** For individuals with impaired immune systems, certain fungal infections, like candida, can be extremely serious or even fatal.[37]**.**It has been discovered that the seeds of *C. papaya* exhibit bacteriostatic activity against a variety of enteropathogens, including *Salmonella typhi,*

*Escherichia coli, Bacillus subtilis, Enterobacter cloacae, Proteases vulgaris, Pseudomonas aeruginosa, and Klebsiella pneumonia*. Gram-negative bacteria were more sensitive to the extract than gram-positive bacteria among those tested[38].

**Antifungal activity**

Numerous bodily organs, including the skin, nails, reproductive system, gastrointestinal tract, heart, and neurological system, can be harmed by fungi[39].The combination of fluconazole and papaya latex inhibits the growth of *Candida albicans*[40].

**Anti-inflammatory activity**

The *C.papaya* leaf extract was examined in rats using edema, granuloma, and arthritis models. The extract showed a significant reduction in paw edema, granuloma formation, and reduced inflammation in rats. Thus it proved the anti-inflammatory activity of C.papaya [41]. Numerous plants or isolated derivatives from the plant source show a role as anti-inflammatory via modulation of various activities [42]. Consuming papaya fruits reduced the anti-inflammatory response in healthy people, which was mediated by regulatory T-cells. [43]. A significant study demonstrated the anti-inflammatory properties of *C. papaya* seeds, and the methanolic extract showed inhibition ranging from 57.1% to 64.2%, which is less than the typical anti-inflammatory medicine aspirin's 85.7%.[ 44,42]. *C. papaya*, as noted above, is a tropical plant containing a wide range of bioactive secondary metabolites (e.g. alkaloids, phenolics, flavonoids, carotenoids, tannins, saponins, etc.) and proteolytic enzymes (papain and chymopapain). Several phytochemicals included in papaya have demonstrated the ability to mitigate chronic inflammatory diseases and their associated negative effects by altering the expression of inflammatory markers.[45]

**Immunomodulatory activity**

Papain, sourced from*C. papaya*, induces human eosinophils to degranulate and produce superoxide anion. The papain activation was eliminated by the E-64 inhibitors, indicating that protease activity is necessary to initiate the eosinophil response. The protein G-linked receptor is most likely the mechanism by which eosinophils carry out this function. Presently, it seems that bromelaine and papain exhibit opposing effects based on the target cell.[46]. Fermented papaya preparation exerts both immunomodulatory and antioxidant activity in the macrophage cell line RAW 264 and it is a macrophage activator, which augments nitric oxide synthesis and TNF-alpha secretion independently of lipopolysaccharides[47].

**Anti-Cancer**

Cancer is one of the most lethal diseases around the world in terms of mortality globally. Lung cancer is the most common cancer in men, followed by breast cancer in women. Phytochemical analysis of papaya suggests that Lycopene, beta carotenoid, benzylisothyocynate, beta-cryptoxanthin, benzyl glucosinolate, chlorogenic acid, caffeic acid, protocatechuic acid, quercetin, and some other significant phytochemicals are present in *C. papaya*. Three classes of bioactive substances—phenolics, carotenoids, and glucosinolates—among the more than 5000 molecules from plants that have been linked to anticancer qualities have drawn a lot of attention in anticancer research. [48]. Researchers discovered that individuals with blood, liver, lung, or stomach cancer have a better chance of life after drinking papaya leaf extract (solution) [49].In vitro studies utilizing papaya seed homogenate extract demonstrated excellent efficiency in decreasing superoxide production and inducing apoptosis in the acute promyelocytic leukemia cell line HL-60. The principal cause of this action was benzyl isothiocyanate, or BTIC [15]. BITC extracted from papaya fruit extract had a cytotoxic effect on developing human colon CCD-18Co cells, causing them to become quiescent. [50].Due to its impact on spindle formation, the aqueous extract of *C. papaya*given to onion bulbs disrupted the mitotic cell division of*Allium cepa*, demonstrating its cytotoxic effect [51]**.**

Papaya (*C. papaya* Linn) seeds include anti-cancer properties that can be used to treat liver and prostate tumors, among other cancers [57]. The goal of this study was to assess the possible cancer-preventive properties of papaya black seeds found in mature papaya fruits. The in vitro cytotoxicity of the "methanolic extract of papaya black seeds" (MPB) 1 against human liver cancer Hep G2 cell lines was studied. The half-maximum inhibitory concentration (IC50) was determined using the 3-(4,5-dimethylthiazoline-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test.

Additionally, Acridine Orange-Ethidium Bromide (AO-EB) labeling has been utilized to assess the apoptotic changes that MPB has generated in cancer cells. Researchers have evaluated gene expression control by targeting the B cell lymphoma-2 (Bcl-2), p53, and caspase-3 genes using quantitative Real-Time Polymerase Chain Reaction (qRT PCR). Our findings indicate that papaya black seeds may be a promising treatment for liver cancer, with an IC50 value of 24.35 μg/mL and the ability to induce apoptosis by upregulating p53 and downregulating Bcl-2 [58].

Amazu et al reported a study using extracts of papaya fruit on Swiss albino male mice, divided into six groups with six animals per group. The mice were fed on mice pellets and had access to food and water. An initial pilot study was conducted to determine the minimum and maximum dosages of a drug extract. The animals were given crude drug extract in different doses, with the negative control group receiving normal saline IP. Symptoms of toxicity or death were observed within 24 hours, and any dead animals were removed. The LD50 was calculated as the probability of the minimum dose that killed half the animals in the study group or the mean of two doses where applicable.[59]

Another study investigated the effects of a methanolic seed extract of *C. papaya* on fresh egg albumin-induced inflammation in rats. The results showed that the extract significantly inhibited edema at all doses, with no significant difference between 200 mg/kg and 100 mg/kg at 60-120 minutes. However, aspirin had a significantly different inhibitory effect, with detectable differences at all periods. The crude extract of C. papaya seeds inhibited inflammation by 57.1% to 64.2%, compared to 85.7% with aspirin. This implies that aspirin has greater anti-inflammatory efficacy than the crude extract, which varied from 57.1% to 64.2%. The study shows that additional characterization and purification of active components may address this difference [59].

**Wound healing**

Traditionally, *C. papaya*has been used to treat wounds and other skin conditions. It is a commonly used, readily available treatment for burns and other wounds, and is particularly popular in developing nations. The study conducted by Nayak et al. evaluated the antibacterial and wound-healing activities of *C. papaya* seed extract. Using an in vivo excision wound model, the ability of an ethanol extract of *C. papaya* seed (50 mg/kg/day) to heal wounds in Sprague-Dawley rats was tested. The animals were randomly divided into four groups of six: Group One was the control group, Group Two received papaya seed extract therapy, Group Three received a 1:1 ratio of mupirocin and papaya seed extract treatment, and Group Four received mupirocin ointment treatment. To evaluate the wound-healing process, the rate of wound contraction and hydroxyproline content were evaluated activity of the seed extract. [52].

**Gastrointestinal health**

The anti-diarrheal efficacy of alcoholic and aqueous extracts from the fruit of *C. papaya* was investigated in albino Wistar rats. When administered orally at doses of 100, 200, and 400 mg/kg, the alcoholic and aqueous extracts showed considerable dose-dependent anti-diarrheal action in cases of magnesium sulfate and castor oil-induced diarrhea. The studied extracts' anti-diarrheal activities were comparable to those of the recommended medicine, loperamide (3 mg/kg). The findings support the traditional use of *C. papaya* in the treatment of diarrheal diseases [53]. The current study aimed to assess the antidiarrheal activities and phytochemical content of *C. papaya* fruit against a variety of gastrointestinal pathogens.Powdered plant components from both raw and ripe fruit were extracted using a Soxhlet system and a range of solvents, including petroleum ether, benzene, chloroform, acetone, ethanol, and water. phytochemical examination of *C. papaya* revealed the presence of proteins, amino acids, carbohydrates, tannins, saponins, alkaloids, phenolic compounds, and phytosterols. To determine the extract'sMIC, sterile 96-well microtitre plates were used. The plant extracts inhibited all of the intestinal pathogens examined. The ripe fruit's acetone extract (0.39 mg/ml) and the raw fruit's chloroform extract (25.0 mg/ml) had the highest antibacterial activity among all the fruit extracts. [54].

**Skin conditions**

Cf6One of the most widely grown plants in the world is the *C. papaya*. Traditional medicine has made use of various parts of the *C. papaya*. However, no research using *C. papaya*leaf (CPL) has been done about skin photoaging. The current study examined the anti-aging properties of *C. papaya*leaf on UVB-irradiated normal human dermal fibroblasts (NHDFs). By scavenging reactive oxygen species (ROS) generation, *C. papaya*leaf significantly suppressed UVB-induced activation of mitogen-activated protein kinases (MAPKs) and activator protein-1 (AP-1) signaling. Additionally, our findings showed that CPL inhibited the breakdown of Type I procollagen by regulating matrix metalloproteinases (MMPs) expression negatively and transforming growth factor-β1 (TGF-β1) activity positively.This led to an increase in the synthesis of Type I procollagen, which is a significant part of the extracellular matrix (ECM). Furthermore, the combination of the two active compounds implies that they were CPL's active compounds. When considered collectively, the findings indicate that *C. papaya*leaf holds promise for additional research and the creation of a cosmetic product to treat a range of skin issues, including photoaging [55]. Synthetic skin care lotions containing aluminum, parabens, butylated hydroxyanisole (BHA), and sodium carboxymethyl cellulose are currently overflowing the market. Method: The goal of this work was to create an herbal skin care lotion with the addition of distilled water, beeswax, liquid paraffin,and borax *C. papaya*extract. Following formulation, it was assessed, and several physicochemical and organoleptic parameters (color, odor, and appearance), as well as PH, viscosity, spreadbility, microbiological growth, irritancy, and ease of removal, were identified and documented. Findings: The created skin care herbal lotion's various assessment criteria matched both the marketed formulation and standard values. In conclusion, customers today are more likely to choose natural cosmetics to prevent unneeded side effects [56].

**Neuroprotective effects**

Chronic inflammatory responses in the central nervous system (CNS) indicate neuroinflammation, a pathogenic condition that dramatically accelerates the onset of neurodegenerative disorders, most notably Alzheimer's and Parkinson's disease [60]. *C. papaya* possesses anti-inflammatory and immunomodulatory properties, making it a prospective treatment for neuroinflammation [61, 62]. The flavonoid-rich components of papaya, particularly its leaves and seeds, have strong anti-inflammatory activities [59]. The antioxidant properties of papaya leaf extract are well known for their potential to prevent lipid peroxidation and boost blood antioxidant capacity [63]. Furthermore, particular compounds found in papaya seed extract have been shown to have therapeutic properties for reducing inflammation.

**Action against oxidative stress**

Oxidative stress, one of the most complex disease causes in the human body, is a damaging state caused by an imbalance of endogenous pro- and antioxidant species. It is also a fundamental biological process that contributes to neurodegeneration in Alzheimer's and Parkinson's disease. We have observed that *C. papaya* contains a high concentration of phenolic chemicals and flavonoids. Flavonoids have been demonstrated to reduce the generation of reactive oxygen species (ROS), boost antioxidant protein expression, improve cerebral blood flow and neuron survival, and reduce apoptosis, amyloidogenic effects, and dopaminergic neuron loss [64]. It has been demonstrated that phenolic acids can alleviate a variety of disorders, including depression, neuroinflammation, apoptosis, memory loss, and excitotoxicity [65].

**Cardiovascular benefits**

*C. papaya* contains bioactive substances such as polyphenols, flavonoids, vitamins, and minerals, and it has been shown to have potential cardiovascular effects. Because of its high potassium concentration and vasodilating characteristics, it may reduce blood pressure. Papaya also contains antioxidants that reduce inflammation and oxidative stress, lowering the risk of atherosclerosis and coronary artery disease[66].

**Action against Thrombocytopenia**

*C. papaya* contains bioactive substances such as polyphenols, flavonoids, vitamins, and minerals, and it has been shown to have potential cardiovascular effects. Because of its high potassium concentration and vasodilating characteristics, it may reduce blood pressure. Papaya also contains antioxidants that reduce inflammation and oxidative stress, lowering the risk of atherosclerosis and coronary artery disease [67].

**Future Prospects**

There are several obstacles in the way of fully investigating bio ceutical potential of*C. papaya,* despite its possible advantages to be used as a functional food. A significant barrier is the paucity of research on the therapeutic benefits of *C. papaya*. `Although some studies have yielded encouraging findings, a thorough investigation of the potential health risks of *C. papaya*is still lacking. Additionally, there is difficulty in standardizing the dosage and preparation of *C. papaya*. The fruit is consumed in various forms, including fresh fruit, juice, and supplements, and the dosage and preparation can vary widely. Lastly, it may be difficult to overcome regulatory obstacles to get permission to employ *C. papaya*as a medicinal or nutraceutical agent. The plant is not currently recognized as a medicine by regulatory agencies, making it difficult to obtain approval for its use in clinical settings.

## CONCLUSIONS

Hence, all the evidence provided in the manuscript about the pharmacological and functional food qualities of *C. papaya* which makes it a viable option for medicinal and nutraceutical uses. An untapped market that is worth more than USD 50 million in 2024 for the multi-usage traditionally important plants like *C. papaya.* The fruit of the plant has many properties like antibacterial qualities that can fight infections, antioxidants that shield cells from harm, and enzymes that help with digestion and decrease inflammation.

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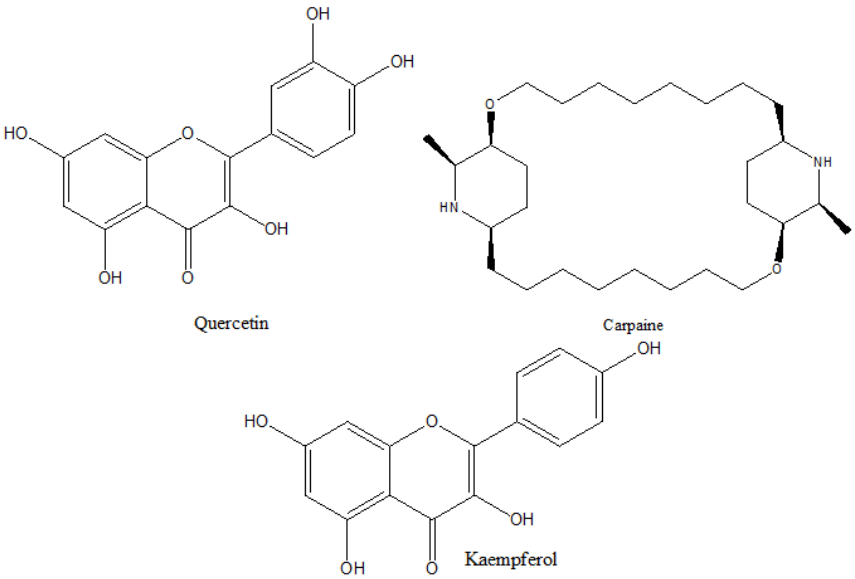


**Fig.1. Phytochemicals present in *C. papaya***

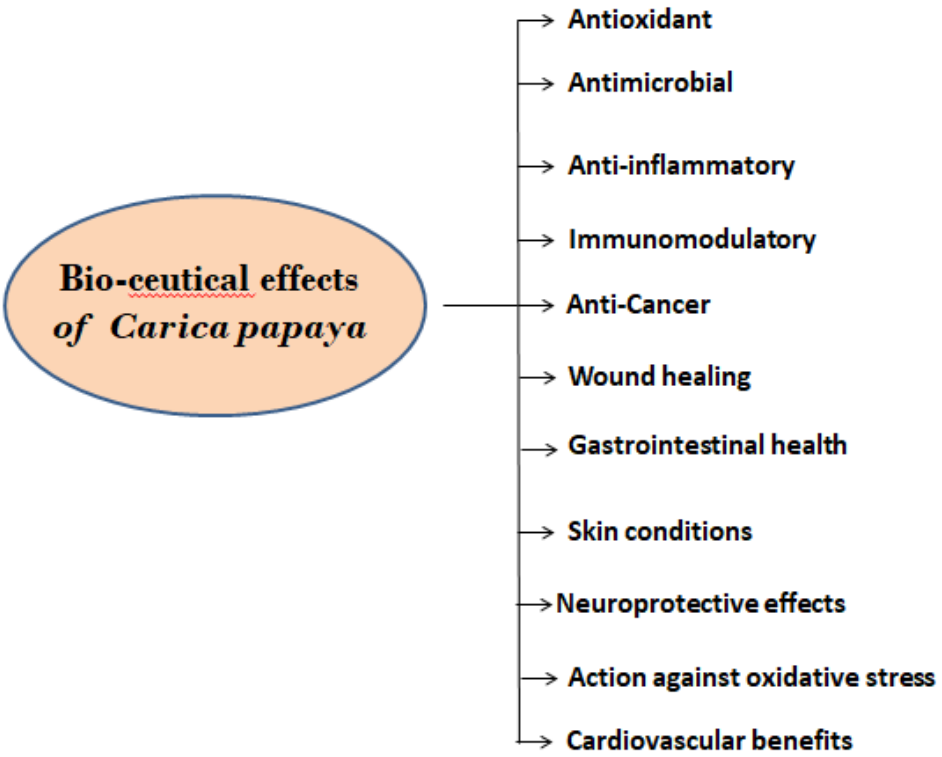


**Fig.2. Bio-ceutical effects of *C. papaya***

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**RESEARCH ARTICLE**

# Prevalence and Distribution of Virulence Determinants in Diarrheagenic *Escherichia coli* associated with Children Under Five Years of Age

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## ABSTRACT

*Escherichia coli*(*E.coli*) is a ubiquitous member of the human gut microbiota, existing as both commensal and pathogenic strains. The pathogenic variants of *E.coli*, known as Diarrheagenic *Escherichia coli*(DEC), pose a significant health threat to children under five globally, particularly in developing regions, where they cause childhooddiarrhea. The objective of the present study was to identify pathogenic variants of *E.coli* from stool samples of children with acute diarrhea (n=144)admitted to pediatric ward of Sardar Patel Medical College (PBM Hospital) and samples from untreated community sewage sources (n=40) collected in the Bikaner district of Rajasthan, India. These samples were processed inculture-based analyses employing established laboratory protocols. For molecular characterization, bothmonoplex and multiplex-PCR analysis targeted 16 virulence genes associated with 6 different DEC pathotypes. These included *stx1*, *stx2*, and *hlyA*associated with Shiga toxin-producing *E. coli* (STEC), which includes the Enterohemorrhagic *E. coli* group (EHEC); *irp2*, *aaiA*, *pic*, *aafA*, *aggR*, and *pCVD432* linked to Enteropathogenic *E. coli* (EACE); *bfpA* and *eae* associated with Enteropathogenic *E. coli* (EPEC); *st*, *lt*, and *est*associated with Enterotoxigenic *E. coli* (ETEC); *ipaH* associated with Enteroinvasive*E. coli* (EIEC); and *daaE* associated with Diffusely Adherent *E. coli* (DAEC). Among the 90 presumptive*E. coli*samples [66 (45.8%) from stool and 24 (60%) from sewage], 58 samples harboured one or more virulence genes associated with specific DEC pathotype. The most common pathotype was observed to be enteroaggregative EAEC, which accounted for 35.7% of cases in children under five and 43.7% of cases in community sewage samples followed by ETEC, and EHEC. The research underscores the widespread

presence of the DEC pathotypes of across various sources, offering crucial insights into their detection, prevalence, and epidemiological patterns within the Bikaner city of Rajasthan in India.

**Keywords**: Childhood-diarrhea, DEC Pathotypes, Virulent genes, Multiplex-PCR.

## INTRODUCTION

*Escherichia coli*is a commensal bacterium that commonly inhabits the human gastrointestinal tract. However, certain pathogenic strains can cause a wide range of diseases, including enteric and diarrheal illnesses [1-4]. Diarrheal diseases are a significant public health concern, particularly in developing countries, and are caused by various pathogens such as bacteria (e.g., *E. coli andSalmonella* spp.), viruses (e.g., rotavirus and norovirus), and parasites (e.g., *Giardia lamblia*) [3,5]. *E.coli*is the most important etiological agent of childhood diarrheal diseases globally, contributing substantially to morbidity and mortality in infants and young children [3,5]. Pathogenic *Escherichia coli* (*E.coli*) infections are estimated to cause approximately 2 million deaths globally each year, and the majority of these deaths, approximately 90%, occur in children under the age of five [6]. Diarrheagenic *E. coli*(DEC) strains are classified based on clinical features, epidemiological characteristics, and the presence of pathogenicity-specific virulence factors. DEC can be categorized into six main pathotypes which include Enteropathogenic *E. coli*(EPEC), Shiga toxin-producing *E. coli* (STEC), which encompasses the enterohemorrhagic *E. coli* group (EHEC), Enterotoxigenic *E. coli*(ETEC), Enteroaggregative *E. coli*(EAEC), Enteroinvasive*E. coli*(EIEC), and Diffusely adherent *E. coli*(DAEC) [1,3,4].The latter EPEC is subdivided into typical EPEC (tEPEC) and atypical EPEC (aEPEC). Each pathotype exhibits distinct geographical distributions and possesses specific virulence markers. Pathogenic strains of *E. coli* can be classified based on genetic sub-structures, which exhibit distinct phylogenetic relationships. These phylogenetic groups (A, B1, B2, and D), known as phylogroups, are characterized by distinct phenotypic and genotypic traits [7].Pathogenic *E. coli* strains possess virulence factors encoded by specific genes located on chromosomes or mobile genetic elements like plasmids and transposons [8,9]. Diarrheagenic *E. coli* (DEC) pathotypes have distinct virulence factor profiles encoded by specific gene clusters. These pathogenic genes encode activities like adhesion, invasion, attachment, iron acquisition, motility, and toxin production. The four major virulence classes are colonization, fitness, toxins, and effectors [7]. Pathogenic strains of *E. coli* are responsible for various diarrheal diseases, each characterized by distinct virulence factors and clinical implications. For example,EAEC is a significant emerging pathogen associated with persistent diarrhea in children in developing countries [10]. Its main characteristic is an aggregative adherence pattern on cultured epithelial cells, mediated by the production of fimbrial colonization factors known as aggregative adherence factors (AAFs)[11]. While ETEC produces heat-labile (LT) and heat-stable (ST) enterotoxins, which cause watery diarrhea[2,3]. EPEC harbors the locus of enterocyte effacement (LEE) pathogenicity island, which is responsible for the attaching and effacing (A/E) phenotype on host enterocytes. EPEC can be classified as 'typical' or 'atypical' based on the presence or absence of the EPEC adherence factor (EAF) plasmid, which comprises the cluster of genes encoding the bundle-forming pilus (*bfpA*). EHEC, also belonging to Shiga toxin-producing *E. coli* (STEC), produces Shiga toxins (Stx1 and Stx2) and can cause large outbreaks. DAEC harbors the dispersin-encoding gene, *daaE*, which facilitates adherence to intestinal epithelial cells [2]. EIEC shares many characteristics with *Shigella* species. A critical virulence factor associated with EIEC is the invasion plasmid antigen H (*ipaH*) gene, which plays a central role in the invasion process. The *ipaH* gene encodes proteins that facilitate the invasion of intestinal epithelial cells[2].

Identification of *Escherichia coli* often relies on traditional laboratory culture methods. However, for characterizing Diarrheagenic *E. coli* (DEC) pathotypes, various molecular methods are employed to detect virulence genes associated with different categories of DEC. These methods include Polymerase Chain Reaction (PCR), DNA hybridization, Multi-Locus Sequence Typing (MLST), ribotyping, and 16S rRNA gene sequencing [12-14]. Among cutting-edge methods, multiplex-PCR assays are particularly effective in detecting multiple virulence factors simultaneously. This technique involves designing primer sets to target specific DNA sequences associated with

virulence factors, allowing for simultaneous amplification of multiple genes in a single reaction. The present study explores the characterization of DEC strains responsible for diarrheal diseases, focusing on their unique virulence factors and clinical implications. It delves into each pathotypes molecular signatures and clinical relevance in diarrheal illnesses, highlighting the role of multiplex PCR assays in identifying DEC pathogenicity.This study emphasizes the need for surveillance and control measures to address diarrheal diseases, especially in children under five years of age. The study enhances understanding of DEC prevalence, distribution, and genetic traits among stool samples of children with acute diarrheaadmitted to pediatric ward of Sardar Patel Medical College (PBM Hospital) and samples from untreated community sewage sources collected in Bikaner, Rajasthan, the northwest region of India. This underscores the importance of ongoing research and public health interventions to combat DEC-related childhood diarrheal illnesses in the country.

## MATERIAL AND METHODS

**Study population and sampling sites**

In this study, a total of 144 stool samples were collected from pediatric outpatients aged 0-60 months suffering from acute diarrhea, who were hospitalized in the pediatric ward of Sardar Patel Medical College (PBM Hospital). This cross-sectional study was conducted from September 2022 to March 2023. The sample size determination was based on the number of children under five years of age admitted to the pediatric ward of SPM medical college during the same period in the previous year (September 2021 to March 2022). Case selection was made by the following inclusion criteria: children under 60 months of age suffering from diarrhea and not having been administered antibiotics.Based on guardian information, children with acute diarrheawere enrolled in the study. The study included children under 5 years of age with acute community-acquired diarrhea, defined as the passage of 3 or more watery and loose stools in 24 hours, with or without clinical symptoms of an enteric ailment such as nausea, vomiting, abdominal pain or cramps, dehydration, fecal urgency, or dysentery. The study population was limited to children of Indian origin residing in the Bikaner city of Rajasthan. Information on acute diarrheal symptoms, including the number of loose/watery stools, presence of bloody or mucoid stools, vomiting, and fever ≥39°C, was collected through parental discussion and medical record review. Other clinical features and risk factors recorded included nausea, abdominal pain, duration of diarrhea, age group, feeding type,and drug use. Based on the WHO classification of dehydration, children with acute diarrhea were categorized as having mild diarrhea (no dehydration) or moderate diarrhea (mild to moderate dehydration). Severe dehydration was considered a medical emergency requiring immediate treatment.The collection of sewage samples, particularly untreated community sewage samples, was conducted from various regions of Bikaner, including both rural and urban areas. A total of 40 samples were collected overfour months, from March 2022 to June 2022. The ease of sample collection was facilitated by the relatively shallow depth of the sites. However, sites with deeper depths posed a challenge, and poles were used to collect samples while adhering to safety protocols to prevent infection.

**Specimen collection and transportation**

The stool and sewage samples were collected in sterile, wide-mouthed, leak-proof containers with tightly fitting lids, labelled with patients' names, ages, genders, and collection months, and stored at the hospital's microbiology laboratory. Subsequently, all samples were transferred to the Microbiology department of Maharaja Ganga Singh University in Bikaner (MGSU), Rajasthan for further processing within 24 hours of receipt.

**Isolation and identification of *E. coli***

The stool and sewage samples were initially inoculated into a selective enrichment medium, lauryl sulphatetryptose broth, at a 1:10 ratio. The inoculated broth was then incubated overnight at 37°C. Furthermore, the samples were sub-cultured onto MacConkey agar and eosin-methylene blue (EMB) agar plates, which were incubated at 37°C for 18-24 hours. MacConkey agar and EMB agar serve as selective and differential media for the isolation and identification of coliform bacteria and *E. coli*. On MacConkey agar, *E. coli* colonies appear as pink to red colonies due to lactose fermentation. EMB agar is more selective, and *E. coli*colonies typically exhibit a characteristic metallic green sheen

with a dark centre.The biochemical tests to characterize *E. coli* were performed [15]. First Gram staining was performed to confirm *E. coli*, *E. coli* is a Gram-negative bacterium, which means it will stain red or pink due to its thin peptidoglycan layer and outer lipid membrane. The IMViC test, a set of four biochemical tests, is commonly used to differentiate *E. coli* from other Enterobacteriaceae. The isolates in this study exhibited positive results for the indole and methyl red tests, while the Voges-Proskauer, citrate, and urease tests produced negative findings [16,17].

**Molecular characterization of diarrheagenic *E. coli***

Genomic DNA was extracted from presumptive *E. coli* to identify DEC specific virulence genes. The process began by suspending each*E. coli* isolate in LB medium separately and after incubation (0.5 optical density at 600nm), centrifuging at 6000 rpm for 10 minutes. The supernatant was discarded, and the pellet was used for DNA extraction.The bacterial cells underwent lysis using a chemical combination which include SET buffer (150 mM NaCl, 1 mM Tris/HCl pH 8.0, and 0.5 mM EDTA), 10% sodium dodecyl sulfate (100 µl), and proteinase K (5 µl), with an incubation period at room temperature for 1 hour. Phenol was introduced to separate the nucleic acid from other cellular substances, followed by centrifugation at 12,000 rpm for 15 minutes. The upper layer was extracted by adding an equal volume of chloroform-isoamyl alcohol (24:1) solution and undergoing centrifugation again at 12,000 rpm for 15 minutes. Afterwards, the supernatant was mixed with an equal volume of ice-cold isopropyl alcohol and centrifuged at 10,000 rpm for 10 minutes, precipitating the DNA with ethanol. The DNA precipitate was then resuspended in 50 µl of Tris-EDTA (TE) buffer. The quality of the bacterial DNA was assessed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide, using 1 × Tris-borate-EDTA (TBE) buffer at 100 V for 45 minutes, followed by examination of the results under a UV transilluminator. This ensured the extraction and purification of high-quality genomic DNA, subsequently utilized for PCR amplification to identify DEC pathotypes.

**Monoplex and Multiplex-Polymerase chain reaction (PCR)**

DNA templates were subjected to monoplex and multiplexPCR with specific primers (Table 1), for the identificationand detection of different DEC pathotypes such as EPEC, ETEC, EHEC, EAEC, EIEC, and DAEC. Monoplex and multiplex PCR primer protocols were employed to acquire, analyse, and evaluate the primer sequences' specificity, sensitivity, and size. The PCR primers were synthesized by Eurofins (Eurofins Genomics India Pvt. Ltd.). Sixteen different target genes were used to detect DEC by monoplex and multiplex PCR include *irp2*, *aaiA*, *pic*, *aafA*,*aggR* and *pCVD432* (EAEC); *bfpA, eae* (EPEC); *stx1*, *stx2*, and *hlyA* (EHEC); *st*, *lt*, and *est* (ETEC); *ipaH* (EIEC), and *daaE* (DAEC) genes as indicated in Table 1. These virulence genes are crucial determinants to identify DEC pathotypes[18,19]. The multiplex PCR was performed with a 50µl reaction mixture containing 2µl of 50ng DNA template and 5µl of 10x PCR buffer, 2µl of 25mM$MgCl_2$, 5µl of a 2.5mM mixture of deoxy nucleoside triphosphate (dNTP), 1µl of 2U/µl of Taq DNA polymerase, 2µl of 10µm concentration of each forward and reverse primers, with the remaining volume supplemented by nuclease-free water. The conditions for the thermal cycling for multiplex PCR were kept as indicated in Table 2.Standardising the multiplex PCR assay included optimising PCR cycling conditions and primer selection to ensure efficient and specific detection of specificDEC pathotypes. The following three multiplex PCR assays were designed to detect specific DEC pathotypes:

Multiplex PCR I:

- EPEC targeting the *eae* gene.
- EHEC targeting the *stx1* gene.
- ETEC targeting the *lt* gene.
- EAEC targeting the *aggR* gene.

Multiplex PCR II:

- EPEC targeting the *bfpA* gene.
- EHEC targeting the *stx2* and *hlyA* genes.
- ETEC targeting the *st* and *est* genes.
- EAEC targeting the *irp2*, and *aaiA* genes.

Multiplex PCR III:

- EIEC targeting the *ipaH* gene.
- DAEC targeting the *daaE* genes.
- EAEC targeting the *pic*, *aafA*, and *pCVD432* genes.
-

The monoplex-PCR wasalso performed with a 50µl reaction mixture containing 2µl of DNA template and 5µl of 10x PCR buffer, 2µl of 25mM$MgCl_2$, 5µl of a 2.5mM mixture of deoxy nucleoside triphosphate (dNTP), 1µl of 2U/µl of Taq DNA polymerase, 2µl of 10µm concentration of each forward and reverse primers, with the remaining volume supplemented by nuclease-free water. The monoplexPCR thermocycling conditions were kept asinitial denaturation at95°C for 2 minutes, 95°C for 30seconds, annealing at 50° for 1 minute, and extension for 72°C for 1.30 minutes,followed by 30 cycles, with the final10-minute extension at 72°C, the reaction wasrun in the thermos PCR system. The amplified PCR products were evaluated by running 5µl on a 2% (wt/vol) agarose gel along with a 100bp DNA ladder as a molecular marker. Ethidium bromide was used to stain the gel, and the DNA bands were visualized under UV light.

**Quality Control**

A positive control and a negative control were included in PCR assays. The positive controls to detect DEC pathotype include: ATCC 35401 to detect ETEC, ATCC 33780 to detect EAEC, ATCC 43893 to detect EIEC, ATCC 35150 to detect EHEC, ATCC 43887 to detect EPEC. Additionally, DH5α used as a negative control.

**Ethical Approval**

The present research study and its protocol were approved by the Institutional Ethics Committee (IEC) of the Maharaja Ganga Singh University of Bikaner. Data collection and analysis were conducted at the PBM Hospital of Sardar Patel Medical College, Bikaner, Rajasthan. The IEC thoroughly reviewed and endorsed the research protocol to ensure its adherence to ethical standards and regulatory requirements.

## RESULTS

In the present study, a total of 184 samples were collected, comprising 144 stool samples from children under five years of age experiencing acute diarrhea and 40 samples from untreated community sewage. The samples underwent culture-based analyses employing established laboratory protocols, including the assessment of morphological features on MacConkey agar and Eosin Methylene Blue (EMB) agar, alongside IMViC(Indole, Methyl red, Voges-Proskauer, Citrate utilization) biochemical tests (positive or negative). The identification of presumptive *E. coli*isolates from the samples yielded an overall positivity rate of 90 (48.91%).Of the positive samples, 66 (45.83%) were obtained from stool samples, while 24 (60%) were derived from sewage sources. The male-to-female ratio among the total sampled population (stool samples, n=144) was approximately 80 (55.55%) to 64 (44.44%), respectively. Children enrolled in the study encompassed diverse age groups, with 77 (53.47%) up to 24 months, 38 (26.38%) aged between 25 and 48 months, and 29 (20.13%) up to 60 months of age (Table 3).Furthermore, the dietary practices of the participating children were delineated, revealing that 18% were exclusively breastfed, 45.8% received a combination of breast milk and formula milk, and 36.1% were fed with solid foods (Table 3).In children with diarrhea (n=144), the clinical symptoms were mainly defined by variations in stool consistency. The present study indicates that diarrhea in children primarily led to watery stools (43%) and loose stools (29.16%), while a smaller percentage presented with bloody stools (11.11%) or mucoid stools (16.6%) (Table 3). In contrast, *E. coli* prevalence in children with diarrhea, indicated a little male predominance, with 54.5% of positive cases being male and 45.4% female, which is similar to the distribution in negative cases (56.4% male and 43.5% female) (Table 3). In terms of age, the proportion of positive cases is highest in children between the ages of 13 and 24 months (30.3%), followed by 0 to 12 months (24.2%) and 49 to 60 months (21.2%). Children between the ages of 37 and 48 months have the lowest positive rates (9%). In terms of feed type, children who were solely breastfed had the lowest percentage of positive cases (9%), while those who were fed solid food had the largest percentage (48.4%). Additionally, there was variation

in the type of stool; among positive cases, watery stools were the most prevalent (48.4%), followed by loose stools (27.2%) and mucoid stools (21.2%). In comparison to negative instances (17.9%), bloody stools were much less common in positive cases (3%) than in negative ones.These findings provide insights into the demographic and nutritional characteristics of the studied population, contributing to a comprehensive understanding of diarrheal disease epidemiology and its associated factors.

Sewage samples were systematically collected from both rural and urban regions within the Bikaner regionas depicted in Table 4. The sampling strategy aimed for inclusivity by encompassing a variety of localities, including slum settlements and affluent residential areas. Geographical categorization facilitated the classification of samples according to their urban or rural origins, enabling a detailed examination of potential microbial contamination across diverse environmental settings. This meticulous sampling approach sought to comprehensively capture the range of sewage characteristics prevalent in the study area, thereby augmenting the reliability and relevance of the study findings. Through a comparative analysis, the prevalence of *E. coli* in various rural and urban community sewage samples was observed. The villages of Udairamsar, Palana, Chak Chani, and Barsnghsar each observed with two positive samples out of the 24 positive samples; these villages accounted for 8.3% of the total positive samples. Several settlements, including Husangsar, Kanasar, Madh, Bachhasar, Kotri, and Kolayat, each observed with one positive sample (4.1%). In rural areas, only one negative sample (6.2%) was observed by each of Bachhasar, Kotri, Kolayat, Madh, Husangsar, and Kanasar. However, the pattern was marginally different in urban areas. Nagnechi and Choukhuti each observed with two positive samples (8.3%) out of the 24 positive samples. A single positive sample (4.1%) was observed by each of the following areas: Satellite, Kothari, Kuchilpura, Railway Colony, Dharnidhar, and Police-line. There were no positive samples in Bhutto ka Chauraha or Pugal-phanta, but there were two negative samples (12.5%) in each. There was one negative sample (6.2%) for each of Satellite, Kothari, Kuchilpura, Railway Colony, Dharnidhar, and Police-line.

To detect various virulence genes, 90 presumptive *E. coli*isolates underwent PCR screening using specific primers designed to target different 16 genes associated withDEC(Table 1) (Fig 2). These virulence genes included *irp2*, *aggR*, *aaiA*, *aafA*, *pic*, *pCVD432*, *bfpA*, *eae*, st*x1*, st*x2*, *hlyA*, *lt*, *st*, *est*, *daaE*, and *ipaH*. DECwere categorized based on the presence or absence of these specific virulence genes in the stool samples collected from diarrheic children and from sewage samples (Fig.2). Among the overall 90 samples, 58(64.4%) were positive for at least one of the targeted virulence genes of *E. coli*. (Table 5).Of the 66 presumptive*E. coli* from stool samples, 42 (63.6%) exhibited the presence of one or more virulence genes. The gene *irp2* exhibited the highest frequency at 26.1%, whereas the genes *aaiA*, *est*, and *pic* had the lowest occurrence, each representing 2.3% of the isolates (Table 5). 36.4 % of *E.coli* isolates were found non-pathogenic, based on the absence of all investigated virulence genes.Among 24 presumptive *E. coli* from untreated community sewage, 16 (66.6%) were observed with at least one of the targeted virulence genes. The predominant gene detected was *irp2* (31.2%), identified in sewage samples, The most common gene was the *irp2* similar to the diarrheal children group. Only 6.2% of the sample was positive for the *aafA*, *cvd432*, *stx1*, and *stx2* gene. In contrast, 33.4% of *E. coli* isolates were observed to be non-pathogenic, based on the absence of any investigated gene. None of the samples tested positive for the *aggR* and *eae*genes in both stool and sewage samples (Table 5).

For characterization of typical Enteropathogenic *E. coli* (EPEC), both *bfpA*(bundle-forming pilus) and*eae*(intimin)genes are needed to be present. However, the present study observed, only *bfpA,*gene characterizing theseDEC pathotype as atypical EPEC.Similarly, in the present study, the presence of *stx1*, *stx2*, and *hlyA* genes characterized Enterohemorrhagic *E. coli* (EHEC) strains whilethe presence of *lt*, *st* and *est* genes characterized Enterotoxigenic *E. coli* (ETEC) strains. Enteroaggregative *E. coli* (EAEC) strains were characterized by *irp2*, *aaiA*, *aafA*, *pic*, and *pCVD432* genes, while Enteroinvasive*E. coli* (EIEC) strains were identified by the presence of the *ipaH* gene, and diffuse adherent*E. coli* (DAEC) strains were identified by the presence of the *daaE* gene.

In this way. a total of 58 presumptive *E. coli*was categorized based on the detection of at least one targeted virulence gene associated with DEC. Notable variations were observed in the distribution of these virulence genes among *E. coli* isolates obtained from stool and sewage samples. The most prevalent genes were observed to be *irp2*, present in

27.5% of the samples, and *st*, observed in 20.6% of the samples, followed by *stx1* (8.6%), *stx2* (6.8%), and *hlyA*(6.8%). Additionally, *lt* (6.8%), *aafA* (5.1%), and *daaE* (5.1%) were also identified. Less frequently observed genes included *bfpA* (3.4%) and several others, such as *aaiA*, *pic*, *pCVD432*, *est*, and *ipaH*, which were detected in 1.7% of the total samples (Fig. 1). In our analysis of total 90 presumptive *E. coli*, a total of 32 (35.5%) isolates exhibited an absence of all tested virulence genes. These observations underscore the intricate nature and variability of DEC pathotypes, emphasising the necessity of evaluating the collective presence of virulence factors to comprehend their pathogenicity effectively.The present study observed that DEC pathotypes in stool samples was more frequent in males (61.9%) than in females (38%) (Table 6). The age-wise distributions of DEC pathotypes were not consistent (Table 6). DEC pathotypes EAEC followed by EHECand ETEC were observed to be more prevalent in all age groups (Table 6). The age-wise prevalence of the virulence gene was 23.8% in 0-12 months, 16.6% in 13-24 months, 33.4% in 25-36 months, 14.2% in 37-38 months, and 11.9% up to 60 months of age, respectively. Regarding the children's clinical symptoms, watery and loose diarrhea (42.8%) and (40.4) respectively were the most common diarrheal consistency. Concerning the feed types, 42.8% and 35.7% of DEC pathotypes were detected from combined breast or formula milk, and solid feed respectively (Table 6).

The predominant DEC pathotypes among the stool samples collected from children with diarrhea were found to be Enteroaggregative *E. coli* (EAEC), and Enterotoxigenic *E. coli* (ETEC), with virulence genes detected namely *irp2* (26.1%), *aafA*(4.7%), *aaiA*(2.3%), *pic* (2.3%), and *st*(21.4%), *lt*(4.7%), *est*(2.3%)] respectively. Followed by DEC pathotype characterized as Enteropathogenic *E. coli* (EPEC) harbouring *bfpA*(4.7%), Enterohemorrhagic *E. coli* (EHEC) [st*x1* (9.5%), *stx2* (7.1%), and *hlyA*(4.7%)], Diffusely Adherent *E. coli* (DAEC) [*daaE*(7.1%)], and Enteroinvasive*E. coli* (EIEC) [*ipaH*(2.3%)]. In community sewage samples, the predominant DEC pathotypes were characterized as EAEC [*irp2* (31.2%), *aafA*(6.2%), *pCVD432* (6.2%)], followed by ETEC [*st*(18.7%), *lt*(12.5%)], EHEC [st*x1* (6.2%), *stx2* (6.2%), and *hlyA*(12.5%)].

In contrast, the collective prevalence of DEC within untreated community sewage was determined to be 66.6%. Notably, in sewage samples from both urban and rural areas, the *irp2* gene had a prevalence of 31.2% (Table 7). The *pCVD432* (6.2%) gene linked to EAEC strains was observed to be exclusively present in sewage samples(Table 7). EAEC emerged as the predominant pathotype identified in both stool and sewage samples. Upon broader geographical analysis within the Bikaner region, the overall positivity rate of virulence genes associated with DEC pathotypes in the untreated community sewage was found to be 68.7% in rural regions and 31.25% in urban areas, respectively.

The present study presents the first comprehensive report on the prevalence of six diarrheagenic *Escherichia coli* strains in the Bikaner region of Rajasthan, India. EAECfollowed by ETECand EHEC were identified as the most predominant pathotypes among children under five years of age and in the untreated community sewage samples.

## DISCUSSION

In India, diarrhea continues to be the primary cause of morbidity and mortality among children under the age of five, resulting in an estimated 2.5 billion deaths annually. Within this statistic, diarrhoeagenic *Escherichia coli* (DEC) is attributed to 30%-40% of cases, representing a significant proportion of diarrhoeal illnesses globally, particularly prevalent in developing nations [35-37]. DEC strains have exhibited diverse virulence traits along with instances of antibiotic resistance, presenting notable concerns within the realm of study. In the present study various phenotypic, biochemical, and molecular assays have been employed for the identification and characterization of DEC pathotypes. For the comprehensive identification of DEC pathotypes, *E. coli* isolates confirmed through biochemical methods were selectively targeted for characterization via monoplex and multiplex PCR assays aimed at identifying their pathotype-specific virulence genes. These assays were tailored to target distinct virulence genes linked with each pathotype, facilitating the simultaneous detection of multiple pathotypes within a single reaction [38-46]. Regional disparities in the prevalence of various pathotypes of DEC are extensively documented [37]. In our study,

we gathered a total of 184 samples from both stool (from children with diarrhea admitted to pediatric ward) and sewage sources. Among these, 45.8% and 60% of the samples, respectively, were identified as presumptive*E. coli* using culture techniques and biochemical tests. This aligns with the findings from previous research. For instance, Ballal *et al.*[47] identified *E. coli* as the predominant pathogen in 46.3% of diarrheal patients. Similarly, a study conducted in Orissa, India, by Samal *et al*. [48], indicated that *E. coli* accounted for 75.5% of bacterial enteropathogens among hospitalized patients, with 13.3% being pathogenic strains. In our investigation, we found that 48.9% of the samples contained *E. coli*, with at least frequency of one isolate in 64.4% of the collected samples.

Using PCR assays, our study reveals a higher prevalence of EAEC and ETEC, followed by EHEC, EPEC, DAEC, and EIEC among children with diarrheaand in communities across Bikaner. Out of 66 stool samples, 42 tested positives for DEC wherein EAEC were the most common pathotype, detected in 35.7% of cases, followed by ETEC (28.5%), EHEC (21.4%), EPEC (4.7%), DAEC (7.1%), and EIEC (2.3%). Among 24 sewage samples, 16 were tested positives for DEC pathotypes. EAEC was detected in 43.7% of samples, while ETEC and EHEC were observed in 31.2% and 25% of samples respectively. A study conducted in South India, which is consistent with our findings, identified EAEC as the most prevalent pathotype among children with diarrhea (14.7%), followed by ETEC and EPEC [49]. The present results also consistent with the previous study[50], wherein 50% of cases of persistent diarrhea were attributed to EAEC infection in India.

In the present study, the enrolled patients exhibited a uniform distribution of sexes. Among the study subjects, there were 80 (55.55%) male and 64 (44.44%) female. The data pertaining to children with diarrhea were further categorized into three age groups wherein group A comprised 77 (53.47%) individuals up to 24 months, group B included 38 (26.38%) individuals aged between 25 and 48 months, and group C encompassed over 29 (20.13%) individuals up to 60 months of age. The distribution of six DEC pathotypes namely EAEC, ETEC, EHEC, EPEC, DAEC, and EIEC was examined across these age groups. Remarkably, EAEC followed by ETEC and EHEC pathotypes were observed to be most dominant, while EPEC, DAEC, and EIEC pathotypes were found to be relatively less frequent.

A total of 22 (37.9%) enteroaggregative *E. coli* (EAEC) were detected in both stool and sewage samples. EAEC, the most recently identified DEC, is known to cause travelers'diarrhea and is the second most common type found in both developed and developing countries [51]. The presence of various serotypes, antibiograms, and virulence gene profiles among the isolates underscores the high genetic diversity of EAEC strains [29].Enterotoxigenic *E. coli* (ETEC) strains hold significance as the predominant strain among pediatric patients, particularly those under five years of age [52]. In our study, ETEC was detected in a total of 17 samples (29.3%). However, findings from a previous study reported a higher prevalence of ETEC among children under 12 months compared to those over 12 months [53]. The children under 2 years old were predominantly affected by EPEC infections [54]. Concurrently, Enteropathogenic *E. coli* (EPEC) accounted for 3.4% of diarrheal disease observed in the present study. Considering the present observation, the findings are noteworthy in two studies: one from northern India (Kashmir) claiming a prevalence of 7.6% [55] and the other from southern India (Mangalore) indicating an EPEC prevalence of 11% [56]. Additionally, the prevalence of Enteroinvasive*E. coli* (EIEC) in stool samples was 1.7%. In comparison, Diffuse adherent *E. coli* (DAEC) which was observed in 5.1% of cases.

## CONCLUSION

The present study revealed a significant prevalence of diarrheagenic *E. coli* pathotypes, particularly EAECfollowed by ETECand EHEC in the Bikaner region of Rajasthan, India with a notably higher incidence among children under five. The detection rates of other DEC pathotypes, such as EPEC, EIEC, and DAEC, were relatively lower than those of EAEC, ETEC, and EHEC. This study represents the first report on the molecular epidemiology of DEC pathotypes in the Bikaner region. The high prevalence of EAEC followed by ETEC, and EHEC infections in children with diarrheaunder five highlights the need for targeted interventions and improved sanitation to reduce the burden of

diarrheal diseases in this age group. The findings also emphasise the importance of molecular methods in accurately identifying DEC pathotypes, as traditional culture-based techniques may not always be sufficient. The use of monoplex and multiplex PCR assays targeting specific virulence genes can enhance the detection of DEC pathotypes and guide appropriate treatment strategies.This study provides valuable insights into the epidemiology of DEC infections in Bikaner and underscores the need for continued surveillance and research to develop effective prevention and control measures against diarrheal diseases in children.

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**Table 1: Oligonucleotide sequences used in the study for selective amplification of DEC associated virulence genes and their amplicon sizes**

| DEC Pathotype | Target genes | Function | Primer sequence (5′-3′) | | Product size (bp) | References |
|---|---|---|---|---|---|---|
| | | | Forward (FP) | Reverse (RP) | | |
| EPEC | *bfpA* | Bundle-forming pilus | TTCTTGGTGCTTGCGTGTCTTTT | TTTTGTTTGTTGTATCTTTGTAA | 385 | 20 |
| | *eae* | Intimin, promotes attachment | CGGAAGCCAAAGCGCACAAGATTA | TGACCAGAAGAAGCATCCACCGAA | 917 | 21 |
| ETEC | *st* | Heat-stable toxin | TTTATTTCTGTATTGTCTTT | ATTACAACACAGTTCACAG | 171 | 22 |
| | *lt* | Heat-labile toxin | AGCAGGTTTCCC | GTGCTCAGATT | 130 | 23 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | ACCGGATCACCA | CTGGGTCTC | | |
| | *est* | Heat-stable enterotoxin | ATTTTTACTTTCT GTATTAGTCTT | CACCCGGTACA AGGCAGGATT | 190 | 24 |
| EAEC | *irp2* | Iron acquisition and virulence | AAGGATTCGCTG TTACCGGAC | TCGTCGGGCAG CGTTTCTTCT | 287 | 25 |
| | *aaiA* | Type VI secretion system (T6SS) | AACCGGAGATGC TGAAACTGCG | GGATTGCCATT AGTAGTCACCA G | 384 | 26 |
| | *aggR* | Aggregative adherence and biofilm formation | CCTAAAGGATGC CCTGATGATAA | TAACGCTGGAC ATGAGATAACC | 663 | 27 |
| | *pic* | Mucin degradation and immune evasion | GGGTATTGTCCG TTCCGAT | ACAACGATACC GTCTCCCG | 1175 | 28 |
| | *aafA* | Aggregative adherence fimbriae | ATGTATTTTTAGA GGTTGAC | TATTATATTGTC ACAAGCTC | 518 | 29 |
| | *pCVD432* | Plasmid-encoded regulatory gene promotes biofilm formation and virulence | CTGGCGAAAGAC TGTATCAT | CAATGTATAGA AATCCGCTGTT | 630 | 30 |
| EHEC | *stx1* | Shiga-toxin-I | TTACAGCGTGTT GCAGGGATCAGT | TTGTGCGTAAT CCCACGGACTC TT | 470 | 21 |
| | *stx2* | Shiga-toxin-II | GGCACTGTCTGA AACTGCTCC | TCGCCAGTTAT CTGACATTCTG | 255 | 31 |
| | *hlyA* | hemolysin A | GCATCATCAAGC GTACGTTCC | AATGAGCCAAG CTGGTTAAGCT | 534 | 32 |
| DAEC | *daaE* | Adhesin, involved in diffuse adherence | GAA CGT TGG TTA ATG TGG GGT AA | TAT TCA CCG GTC GGT TAT CAG T | 542 | 33 |
| EIEC | *ipaH* | Host cell invasion | GTTCCTTGACCG CCTTTCCGATACC GTC | GCCGGTCAGCC ACCCTCTGAGA GTAC | 600 | 34 |

**Table 2:** PCR Cycling conditions for multiplex PCR (mpPCR)

| **PCR steps** | **Reagent of the PCR** | **Initial denaturation** | **Denaturation** | **Annealing** | **Extension** | **Final extension** |
|---|---|---|---|---|---|---|
| mpPCR I | PCR buffer:10x<br>$MgCl_2$: 25mM<br>dNTPs: 250µM<br>Taq polymerase: 2U/µl<br>FP and RP: 0.4µm each | 90°C-95°C<br>2 min. | 95°C<br>30 sec. | 50°C-55°C<br>1 min.<br>30 cycles | 72°C<br>1:30 min. | 72°C<br>10 min. |
| mpPCR II | PCR buffer:10x<br>$MgCl_2$: 25mM<br>dNTPs: 250µM | 90°C-95°C<br>2 min. | 95°C<br>30 sec. | 42°C-48°C<br>1 min. | 72°C<br>1:30 min. | 72°C<br>10 min. |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Taq polymerase: 2U/µl<br>FP and RP: 0.4µm each | | | 30-35 cycles | | |
| mpPCR III | PCR buffer:10x<br>$MgCl_2$: 25mM<br>dNTPs: 250µM<br>Taq polymerase: 2U/µl<br>FP and RP: 0.4µm each | 90°C-95°C<br>2 min. | 95°C<br>30 sec. | 50°C-55°C<br>1 min.<br>30 cycles | 72°C<br>1:30 min. | 72°C<br>10 min. |

**Table 3: Distribution of *E. coli* in children with diarrhea based on age, sex, and clinical features**

| Variables | Categories | No. of positive % (n=66) | No. of negative% (n=78) |
|---|---|---|---|
| Sex | Male | 36(54.5) | 44(56.4) |
| | Female | 30(45.4) | 34(43.5) |
| Age | 0-12 months | 16(24.2) | 19(24.3) |
| | 13-24 months | 20(30.3) | 22(28.2) |
| | 25-36 months | 10(15.1) | 12(15.3) |
| | 37-48 months | 06(9) | 10(12.8) |
| | 49-60 months | 14(21.2) | 15(19.2) |
| Feed type | Breastfeed | 06(9) | 20(25.6) |
| | Breastfeed and formula milk | 28(42.4) | 38(48.7) |
| | Solid food | 32(48.4) | 20(25.6) |
| Stool type | Loose | 18(27.2) | 24(30.7) |
| | Watery | 32(48.4) | 30(38.4) |
| | Bloody | 2(3) | 14(17.9) |
| | Mucoid | 14(21.2) | 10(12.8) |

**Table 4: Occurrence of *E. coli* in untreated community sewage samples from urban and rural areas**

| **Rural Sites** | **Latitude** | **Longitude** | **No. of positive (%) n=24** | **No. of negative (%) n=16** |
|---|---|---|---|---|
| **1.** Udairamsar | 27.94006 | 73.30384 | 2(8.3) | 0 |
| **2.** Bachhasar | 27.95756 | 73.19093 | 1(4.1) | 1(6.2) |
| **3.** Palana | 27.84395 | 73.26208 | 2(8.3) | 0 |
| **4.** Chak Chani | 27.87696 | 73.00800 | 2(8.3) | 0 |
| **5.** Kotri | 27.86039 | 72.95937 | 1(4.1) | 1(6.2) |
| **6.** Kolayat | 27.83491 | 72.95611 | 1(4.1) | 1(6.2) |
| **7.** Madh | 27.85667 | 72.93063 | 1(4.1) | 1(6.2) |
| **8.** Husangsar | 28.15676 | 73.41487 | 1(4.1) | 1(6.2) |
| **9.** Barsnghsar | 27.81985 | 73.19961 | 2(8.3) | 0 |
| **10.** Kanasar | 28.13366 | 73.31064 | 1(4.1) | 1(6.2) |
| Urban Sites | | | | |
| **11.** Satellite | 28.02030 | 73.30385 | 1(4.1) | 1(6.2) |
| **12.** Kothari | 28.02636 | 73.29809 | 1(4.1) | 1(6.2) |
| **13.** Nagnechi | 27.99352 | 73.33565 | 2(8.3) | 0 |
| **14.** Choukhuti | 28.02352 | 73.30927 | 2(8.3) | 0 |

| | | | | |
|---|---|---|---|---|
| **15.** Kuchilpura | 28.02272 | 73.31440 | 1(4.1) | 1(6.2) |
| **16.** Bhutto ka chauraha | 28.03075 | 73.31459 | 0 | 2(12.5) |
| **17.** Railway colony | 28.03130 | 73.30124 | 1(4.1) | 1(6.2) |
| **18.** Dharnidhar | 28.00610 | 73.29435 | 1(4.1) | 1(6.2) |
| **19.** Police-line | 28.02785 | 73.31314 | 1(4.1) | 1(6.2) |
| **20.** Pugal-phanta | 28.02733 | 73.29853 | 0 | 2(12.5) |

**Table 5:** Distribution of virulence genes in *E. coli* isolates from stool samples and sewage samples.

| Virulence genes detected | Stool samples from children (%) n=42 | Sewage samples (%) n=16 | Total (%) |
|---|---|---|---|
| *irp2* | 11(26.1) | 5(31.2) | 16(27.5) |
| *aafA* | 2(4.7) | 1(6.2) | 3(5.1) |
| *aaiA* | 1(2.3) | 0 | 1(1.7) |
| *pic* | 1(2.3) | 0 | 1(1.7) |
| *pCVD432* | 0 | 1(6.2) | 1(1.7) |
| *aggR* | 0 | 0 | 0 |
| *bfpA* | 2(4.7) | 0 | 2(3.4) |
| *eae* | 0 | 0 | 0 |
| *stx1* | 4(9.5) | 1(6.2) | 5(8.6) |
| *stx2* | 3(7.1) | 1(6.2) | 4(6.8) |
| *hlyA* | 2(4.7) | 2(12.5) | 4(6.8) |
| *st* | 9(21.4) | 3(18.7) | 12(20.6) |
| *lt* | 2(4.7) | 2(12.5) | 4(6.8) |
| *est* | 1(2.3) | 0 | 1(1.7) |
| *daaE* | 3(7.1) | 0 | 3(5.1) |
| *ipaH* | 1(2.3) | 0 | 1(1.7) |

**Table 6: Distribution of DEC pathotypes with clinical features and risk factors in children with acute diaarhea**

| **DEC Pathotypes and detected virulence genes** | **Variables** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Sex** | | **Age (Months)** | | | | | **Type of Diarrhea** | | | | **Type of feed** | | |
| | **Male** | **Female** | **0-12** | **13-24** | **25-36** | **37-48** | **49-60** | **Loose** | **Watery** | **Bloody** | **Mucoid** | **Breastfeed** | **Breastfeed & Formula** | **Solid** |
| EAEC (n=15) | 9 | 6 | 3 | 4 | 5 | 2 | 1 | 6 | 7 | 1 | 1 | 4 | 7 | 4 |
| *irp2* | 7 | 4 | 1 | 1 | 3 | 1 | 1 | 3 | 2 | - | 1 | 1 | 3 | 2 |
| *aafA* | 1 | 1 | - | 1 | 1 | - | - | 1 | 1 | 1 | - | 1 | 1 | 1 |
| *aaiA* | 1 | - | 1 | 1 | 1 | 1 | - | 1 | 2 | - | - | 1 | 2 | 1 |
| *pic* | - | 1 | 1 | 1 | - | - | - | 1 | 2 | - | - | 1 | 1 | - |
| EPEC (n=2) | 1 | 1 | 1 | - | 1 | - | - | 1 | 1 | - | - | - | 1 | 1 |
| *bfpA* | 1 | 1 | 1 | - | 1 | - | - | 1 | 1 | - | - | - | 1 | 1 |
| EHEC (n=9) | 6 | 3 | 3 | 1 | 2 | 2 | 1 | 3 | 4 | 2 | - | 2 | 4 | 3 |
| st*x1* | 3 | 1 | 1 | 1 | 1 | - | 1 | 1 | 1 | 1 | - | 1 | 2 | 1 |
| st*x2* | 2 | 1 | 1 | - | 1 | 1 | - | 1 | 2 | - | - | 1 | 1 | 1 |
| *hlyA* | 1 | 1 | 1 | - | - | 1 | - | 1 | 1 | 1 | - | | 1 | 1 |
| ETEC (n=12) | 7 | 5 | 3 | 2 | 4 | 1 | 2 | 5 | 4 | 1 | 2 | 3 | 4 | 5 |
| *st* | 5 | 4 | 1 | 1 | 2 | - | 2 | 3 | 1 | 1 | 1 | 1 | 2 | 2 |
| *lt* | 1 | 1 | 1 | 1 | 1 | 1 | - | 2 | 2 | - | 1 | 1 | 1 | 3 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *est* | 1 | - | 1 | | 1 | | | - | 1 | - | - | 1 | 1 | - |
| DAEC (n=03) | 2 | 1 | - | - | 1 | 1 | 1 | 1 | 2 | - | - | - | 2 | 1 |
| *daaE* | 2 | 1 | - | - | 1 | 1 | 1 | 1 | 2 | - | - | - | 2 | 1 |
| EIEC (n=01) | 1 | - | - | - | 1 | - | - | 1 | - | - | - | - | - | 1 |
| *ipaH* | 1 | 1 | - | - | 1 | - | - | 1 | - | - | - | - | - | 1 |
| Total (n=42) | 26 | 16 | 10 | 7 | 14 | 6 | 5 | 17 | 18 | 4 | 3 | 9 | 18 | 15 |

**Table 7: Distribution of DEC pathotypes in sewage samples collected from rural and urban areas**

| Virulence genes | No. of virulence genes from sewage samples (Rural areas) | No. of virulence genes from sewage samples (Urban areas) |
|---|---|---|
| **EAEC (n=7)** | 5 | 2 |
| ***irp2*** | 3 | 2 |
| ***aafA*** | 1 | - |
| ***pcvd432*** | 1 | - |
| **EHEC (n=4)** | 3 | 1 |
| ***stx1*** | 1 | - |
| ***stx2*** | 1 | - |
| ***hlyA*** | 1 | 1 |
| **ETEC (n=5)** | 3 | 2 |
| ***st*** | 2 | 1 |
| ***lt*** | 1 | 1 |
| **Total (Totaln=16)** | 11 | 5 |

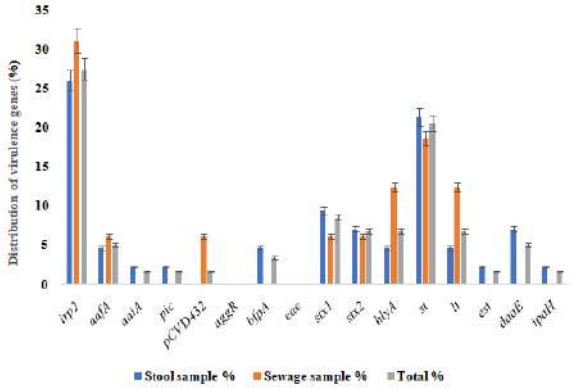


**Fig. 1: Distribution of virulence genes (%) in DEC pathotypes among stool and sewage samples**

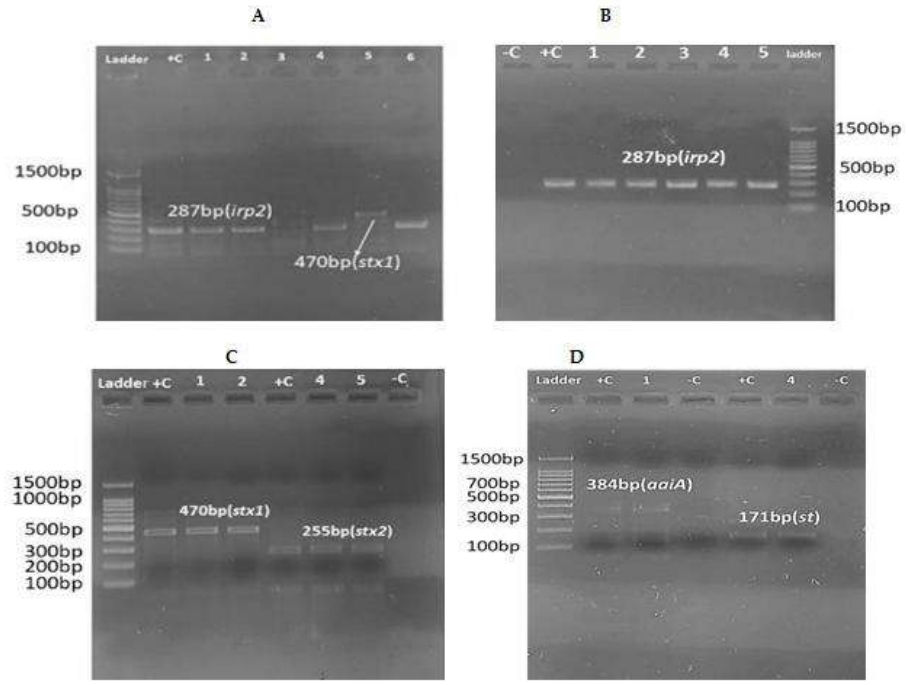


**Fig.2. Multiplex PCR to detect virulence genes in Diarrheagenic *Escherichia coli*: (A) Lane 1; molecular weight marker (100 bp ladder), Lane 2; positive control EAEC: ATCC 33780 (*irp2*), Lane 3,4,6,8; EAEC (*irp2* amplicon size 287bp), Lane 7; EHEC (*stx1* amplicon size 470bp). (B) Lane 1; Negative control, Lane 2; positive control EAEC: ATCC 33780 *(irp2)* and Lane 3 to 7; EAEC (*irp2* amplicon size 287bp), Lane 8; Molecular weight marker (100 bp ladder),.(C) Lane 1; molecular weight marker (100 bp ladder), Lane 2; positive control EHEC: ATCC 35150 *(stx1)*, Lane 3,4,5.6 EHEC (*stx1* amplicon size 470bp, and st*x*2 amplicon size 255bp), Lane 8; Negative control (D) Lane 1; molecular weight marker (100 bp ladder), Lane 2; positive control EAEC: ATCC 33780 *(aaiA)*, Lane 3; EAEC *aaiA*amplicon size 384bp), Lane 4; negative control (DH5*α*), Lane 5; positive control ETEC: ATCC 35401 *(st)*, Lane 6; ETEC (*st* amplicon size 171bp).**

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**RESEARCH ARTICLE**

# A Study of Proximate, Mineral and Phytochemical Properties of Banana (*Musa* spp.) Pseudostem Waste

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## ABSTRACT

Banana pseudostem has most important role in food industries due to its medicinal value, rich in mineral content and easy processibility. The present research study focused on proximate, physicochemical characterization and mineral analysis of Mahalaxmi, Robusta and Grand Naine banana pseudostem. The research study shows that all the three different cultivars contain high amount of moisture content (95%). Banana pseudostem is a high cellulosic and low lignin content lignocellulosicbiowaste. Among the three different banana pseudostem, Mahalaxmi banana pseudostem contain maximum cellulosic content (47.6%) and lowest lignin content (7.7%). The mineral content such as sodium, potassium, calcium, magnesium, phosphorous, manganese, iron and zinc content were estimated. The highest mineral content, calcium (1484.01 mg%), followed by potassium (869.86 mg%) and magnesium (260.51 mg%) were found in Mahalaxmi banana pseudostem as compared to Robusta and Grand Naine banana pseudostem. The WHC (19.3gm of water/g of dry substrate)and OHC (9.3 gm of oil/g of dry substrate) was found maximum in Robusta banana pseudostem. The maximum swelling power (9.3 (g of swollen particles/ g of dry substrate)obtain in Mahalaxmi banana pseudostem. The phytochemical analysis shows that the Alkaloid, Tannin, Flavanoid, Phytate and Protein content is present in Mahalaxmi, Robusta and Grand Naine banana pseudostem. However, the phytochemical study shows that no significant difference was observed in Mahalaxmi, Robusta and Grand Naine banana pseudostem.

**Keyword:** Banana pseudostem, proximate analysis, mineral content and phytochemical analysis.

# INTRODUCTION

Banana is one of the tallest monocotyledons herbaceous flowering plant of the genus *Musa.* Banana plant has belonged to the *Musaceous* family. Banana is the oldest and most economically important cultivated crop in the world. Carl Linnaeus in 1753 first named the genus *Musa*. Banana (genus *Musa*) are the second most important tropical and staple food crop in the world after oil palm[1]. Banana pseudostem have high amount of holocellulose, ash content and low level of lignin content which is very important role in papermaking[2]. Various studies show that unripe banana is a rich source of starch, which offer beneficial effects for the human health. The banana plant contains long overlapped leafstalk. Banana plat is grow in 130 countries of the world. In the World and India, 48.9 and 32 million tons of banana produce. In Gujarat, banana is cultivated in area of 61.9 thousand hector with an annual production of 3779.6 metric ton. Banana is a fourth most important food after paddy, wheat and milk product, in terms of gross value. Banana is a rich source of minerals, carbohydrate, potassium, phosphorous, calcium, iron, vitamin B and C. The central core of the banana is used as a vegetable in various South Indian cuisines.

Banana plant contain root, pseudostem, leaf and fruit. Banana is a monocotelidious plant, it produces only single bunch of banana fruit during the life cycle. After harvesting the fruit of banana, the stem is cut and left on the soil plantation which create the hazardous environmental problems. This agricultural rich lignocellulosic waste can be used in the in paper making, pulp, high quality dress material, cardboard, teabags, and currency notes[3,4]. The aim of this paper is to study the proximate, mineral and physico-chemical properties of Mahalaxmi, Robusta and Grand Naine banana pseudostem.

# MATERIALS AND METHODS

## Materials

Three cultivar of banana (Grand Naine, Robusta and Mahalaxmi) were collected from local banana farm, Anand, Gujarat, India. Cetyltrimethyl ammonium bromide (CTAB), silver nitrate, potassium acetate, ferric nitrate, oxalic acid, hydrochloric acid, Sulphuric acid, ethyl alcohol, tertiary butyl alcohol, acetone, sodium lauryl sulfate, ethylene diaminetetraacetic acid (EDTA), borax, disodium phosphate, ethylene glycol, glacial acetic acid (GAA), copper sulfate, folin's reagent, all the chemicals were purchased from Himedia, Mumbai, India.

### Banana pseudostem collection

The leaf sheaths were separated by cutting the banana pseudostem with the help of the knife. The soil particles were removed by washing treatment and dried in a hot air oven at 85°C for 5-6 h. The dried sheaths of pseudostem were ground to obtain 3-5 mm in length particle size substrate. The chemical analysis of banana pseudostem moisture, lignin, cellulose, hemicellulose, carbohydrate, protein, crude fat, and crude fibers) was evaluated.

#### Compositional analysis

The compositional analysis of three different varieties of banana pseudostem was evaluated according to method described by Van Soest*et al*., (1991)[5]. 1 gm of banana pseudostem substrate was mixed with 100 ml of ADS (acid detergent solution). ADS contains 20 g/l cetyltrimethyl ammonium bromide (CTAB) and 1N sulfuric acid (v/v). The banana pseudostem substrates were refluxed with the ADS for 1 hr. The treated substrate was filtered through the sintered glass crucible. The filtrated substrate was washed with warm distilled water followed by ethanol. The residues were dried at 110°C for 24 h and weighed to calculate the weight of ADF (acid detergent fibers).

$$ADF(\%) = \frac{Weight\ of\ crucible\ and\ acid\ treated\ fibers - Weight\ of\ crucible}{Initial\ weight\ of\ the\ substrate} X100$$

The lignin content of banana pseudostem substrate was determined by a mixture of permanganate solution. ADF were treated with potassium permanganate solution [potassium permanganate (50 g/l) and silver sulfate (0.05g/l)] and the buffer solution which contain 40% (v/v) tertiary butyl alcohol, 50% (v/v) GAA, 0.5% (w/v) potassium acetate, 0.015% (w/v) silver nitrate and 0.6% (w/v) ferric nitrate for 90 minutes. The demineralized solution (a mixture of 5% (w/v) oxalic acid, 5% (v/v) hydrochloric acid and 70% (v/v) ethanol) was used to treat the residue, to obtain the white fibers. The demineralized fibers were treated with warm distilled water and dried at 110°C for 24 h. The lignin content was calculated by using the following equation. The content of ash was evaluated by using muffle furnace at 500°C ±5°C for 6 hours. The remaining substrate residues were considered as an ash content and the loss in weight corresponded to the cellulosic amount.

$$Lignin(\%) = \frac{Weight\ of\ ADF\ residues - Weight\ of\ demineralised\ residue}{Weight\ of\ the\ initial\ residue} X100$$

The natural detergent fiber (NDF) was used to evaluate the content of hemicellulose of banana pseudostem. 1 gm of substrate was mixed with the NDF solution. The NDF solution contain 1% (w/v) sodium lauryl sulfate, 1.9% (w/v) ethylenediamine tetra acetic acid, 0.7% (w/v) borax, 1% (w/v) disodium phosphate and 1% (v/v) ethyl glycol added to avoid the formation of foam and maintain the neutral pH. The NDF solution and banana pseudostem substrate was refluxed for 1 hr. Thereafter, the treated substrate was washed with warm distilled water followed by 95% ethanol (v/v). The residues were filtered with the help of glass crucible, dried overnight at 110°C and weighed to calculate natural detergent fiber.

$$NDF(\%) = \frac{Weight\ of\ crucible\ and\ NDF\ residues - Weight\ of\ crucible}{Initial\ weight\ of\ the\ residue} X\ 100$$

The hemicellulosic content of banana pseudostem is calculated using the following equation
Hemicellulose (%) =Weight of NDF residue (%) −Weight of ADF residue (%)

**Moisture content and dry matter**

The moisture content and dry matter of three different varieties of banana were evaluated by the oven dry method according to Wang *et al*., (2016)[6]. The initial weight of the banana pseudostem were recorded and the samples were dried in the oven at 105°C until constant weight obtained. The moisture content was calculated by the percentage difference between initial and final weight of the banana pseudostem. The remaining residues were considered as a dry matter.

$$Moisture\ (\%) = \frac{Initial\ weight\ of\ the\ sample - Final\ weight\ of\ the\ sample}{Initial\ weight\ of\ the\ sample} X\ 100$$

**Extractive and Crude fat**

The extractive of Mahalaxim, Robusta and Grand Naine banana pseudostems were determined according to TAPPI T 204 cm 97 (1997)(Pereira*et al*., 2010)[7]. Crude fat was examined by petroleum ether extraction method using soxhlet apparatus. The crude fat content was calculated by the following equation.

$$Crude\ fat = \frac{Final\ weight\ of\ the\ residues - Initial\ weight\ of\ the\ residues}{Initial\ weight\ of\ the\ residues} X\ 100$$

**Mineral analysis**

The determination of mineral content of three cultivar of banana were evaluated by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) perkin Elmer, USA, Avio 200. **.7**

**Swelling power and solubility of banana pseudostem**

The swelling power and solubility of banana pseudostem was examined according to slightly modified method described by Aziz *et al*., (2011)[8]. 1 gm of banana pseudostem substrate was mixed with 20 ml of distilled water. The reaction system was stirred continuously on the magnetic stirrer at 90°C for 30 minutes. The mixture was centrifuged at 8000 rpm for 10 minutes. The supernatant was transferred into the petri plate and dried in the oven at 110°C, the pellet residues were weighed and considered as a swollen granule. The following equation was used to determine the solubility and swelling power of three cultivars of banana.

$$Solubility = \frac{Weight\ of\ dried\ residues}{Initial\ weight\ of\ the\ residues} X\ 100$$

$$Swelling\ power = \frac{Weight\ of\ swollen\ granules}{Initial\ weight\ of\ the\ residue - Solubility} X100$$

**Water and oil holding capacity**

The water holding capacity (WHC) and oil holding capacity (OHC) of three different cultivars of banana pseudostems were examined according to method described by Yadav *et al*., (2016)[9]. The WHC and OHC are very important parameter as it defines the quality of the food. 1 gm of substrate was mixed with 25 ml of water and olive oil to estimate the WHC and OHC. The reaction mixture was stirred continuously on the magnetic stirrer at room temperature for 30 minutes. The suspension was centrifuged at 8000 rpm for 10 minutes and weighed the residues. Water and oil holding capacity of banana pseudostem was calculated and expressed as a gram water and oil per gram of substrate.

**Quantitative phytochemical analysis**

Phytochemical such as flavonoid, alkaloid, phytate, tannin and phenolic impart color to vegetable and fruits. The phytochemicals play important role in antioxidant property and other physiological functions. Alkaloid content was analyzed by mixing 5 gm of substrates with 200 ml of 20% HCL (v/v). The reaction mixtures were incubated for 4 hours at 25°C. After the 4 h of incubation, the reaction system was filtered using whatmann filter paper No.42. The concentrated ammonium hydroxide was added drop wise in to the concentrated filtrates until the precipitate was complete. The precipitates were collected and rinsed with two time diluted ammonium hydroxide. The residues were collected on the pre-weighed filter paper. The residues were dried in the oven at 80°C. The amount of alkaloid was calculated using the following equation.

$$Alkaloid(\%) = \frac{Weight\ of\ filter\ paper\ and\ residues - Weight\ of\ filter\ paper}{Weight\ of\ sample} X\ 100$$

Flavonoid is a polyphenolic water soluble compound present in the plant kingdom as secondary metabolites (Chávez-González *et al*., 2020)[10]. The estimation of flavonoid was done by Aluminum chloride method described by Al Amri and Hossain (2018)[11]. The different concentration (10 µg/ml-100 µg/ml) of banana pseudostem substrates were prepared by adding the methanol. The reaction system was mixed with 125 µl water and 75 µl 5% (w/v) sodium nitrate. The reaction system was mixed properly and incubated for 6 min. After incubation, 150 µl of 10% (w/v) aluminum chloride was added and incubated for 5 min. After 5 min. of incubation, 500 µl of 4% (w/v) sodium hydroxide and 275 µl water were added. The absorbance was recorded at 510 nm against black by using SHIMARDZU 1800 UV-Vis spectroscopy. The content of flavonoids was determined concerning the standard curve quercetin. The flavonoid content is expressed in µg of QE/g of banana pseudostem sample.

The tannin content of banana pseudostem was quantified using the method described by Bello*et al*., (2013)[12] with slight modification. 5 gm of substrate was mixed with 100 ml of petroleum ether and kept at room temperature for 24 h. Thereafter, the samples were filtered and petroleum ether was evaporated. The 5 ml filtrates were then mixed with

2 ml of 0.1 M $FeCl_3$ in 0.1 N HCL and 8 mM potassium ferrocyanide. The absorbance was recorded using the spectrometer (SHIMARDZU UV-1800) at 725 nm. The amount of tannin was calculated using the following equation.

$$Tannin\ (\%) = \frac{Abs\ of\ the\ sample\ X\ tannin\ conc.\ in\ standard}{Weight\ of\ initial\ sample} X\ 100$$

The estimation of phytate content in banana pseudostem was done by adding 2% (v/v) HCL (100 ml) with 0.2 gm of pseudostem substrate. The solutions were kept for 3 h at room temperature. Thereafter the solutions were filtered and 50 ml of filtrates were mixed with 100 ml of distilled water. This solution was titrated against $FeCl_3$ with 0.3% (w/v) ammonium thiocynate (10 ml) as an indicator until a brownish yellow color appeared. The phytate concentration was calculated using the following equation.

$$Phytic\ acid = \frac{Titrate\ value\ X\ 0.00195\ X\ 1.19 X\ 100}{Initial\ weight\ of\ sample}$$

Where, 0.00195 is a gm of iron per ml and 1.19 is the mg of phytin phosphorous per ml.

Phenolic content was evaluated by Folin-Ciocalteu test using the method described by Molina-Cortes *et al*., (2019)[13]. 0.4 ml of the extracted samples of three different banana pseudostems were mixed with 4 ml of distilled water followed by 0.4 ml of Folin-Ciocalteu reagent. The solutions were incubated for 10 min. at room temperature in dark condition. Thereafter, 4 ml of Sodium carbonate (7% w/v) was added into the reaction system and the solutions were mixed properly and incubated for 90 minutes at room temperature in the dark environment. Gallic acid (GA) is used as a standard and distilled water is used as a blank. The absorbance was recorded in spectrophotometer (SHIMARDZU UV 1800) at the wavelength 730 nm. The phenolic content was expressed as mg equivalent of gallic acid per gm of banana pseudostem sample (mg GAE/g).

## RESULTS AND DISCUSSION

**Proximate analysis**

The proximate composition of three different varieties of lignocellulosic banana pseudostem (Robusta, Grand Naine and Mahalaxmi) is shown in Table 1. The chemical composition of banana pseudostem varies with the species of banana pseudostem, age of plant, plantation soil and environmental condition[14,15]. Cellulose, hemicellulose and lignin are the main constitutes of banana plant cell wall[16].

Among these plant fibers, lignin is a very strong component which cannot be easily digested. Cellulose is a major constituent of banana plant followed by hemicellulose and lignin. The result shows that the banana pseudostem is a rich source of cellulose. The highest cellulosic content was found in species of Mahalaxmi (47.6%) followed by Grand Naine (42.7%) and Robusta (40.9%). Cellulose is a main component of the primary and secondary cell wall of banana pseudostem due to that banana plant is highly cellulosic in nature. Similar results were also observed by many researchers. Jayaprabha*et al*., (2011)[14] reported 46.3% cellulose in Nendran banana plant. Preethi and Balakrishna (2011)[17] reported 48.19% cellulose in Grand Naine banana pseudostem. Viswanathan*et al*., (1989)[18] reported the 35.9% cellulose content in Robusta which is 5% lower value than our research study. The variation in the lignocellulosic content may be due to the climate and soil.

Hemicellulose is a second most important component in the banana pseudostem. Hemicellulose content was found in the range of 23% - 29% in banana pseudostem. The highest hemicellulosic content found in the Mahalaxmi banana pseudostem (29.4%) followed by Robusta (28.9%) and Grand Naine (23.3%). Similar results were also observed by Viswanathan et al., (1989)[18] for Robusta. They found 21.9% hemicellulose in stalk of *Musa cavendishi.* Banana plant have lower lignin content as compared to other natural fibers such as jute and coir [15].

Mahalaxmi banana plant contains 7.7% lignin, Robusta contain 9% lignin and 8.8 % lignin found in Grand Naine. Poyyamozhi and Kadirvel (1986)[19] reported the lowest lignin component 9-10% in Robusta. Viswanathan et al., (1989) [18] also found the 9.4 % lignin component in Robusta species. The lignin content of Mahalaxmi, Robusta and Grand Naine is lower than the lignin content of *M. acuminate* Colla (12.7%), as reported by Cordeiro *et al*., 2004[20]. Shivashankar*et al*., (2006)[3] found 2.2-time higher lignin content in Robusta (20.23%). The ash content was found in the range of 2.7% - 4.5%. The highest ash content was observed in Mahalaxmi banana plant (4.5%) and the lowest in Grand Naine (2.7%). The evaluation of ash content indicates that the Mahalaxmi banana plant contain high mineral level than the Robusta and Grand Naine. Jayaprabha*et al*., (2011)[14] found 2.8% ash content in pith removed pseudostem and 10.7% ash content in whole pseudostem sheaths. The ash content of all the three different varieties of banana pseudostem in the present study is similar to the ash content of the *Musa cuminata*x balbisianaColla (3%), as reported by Aziz *et al*., (2011)[8].

**Moisture content and dry matter**

Table 1 shows the moisture level and dry matter of Mahalaxmi, Robusta and Grand Naine banana pseudostem. Three different cultivars of banana pseudostem have moisture content in the range of 94% - 96%. No significant difference was observed in the moisture content of three different varieties of banana pseudostem. High moisture level favors the quality and yield of the banana pseudostem fiber. Shivashankar*et al*., (2006)[3]reported 93.2% - 94.6% moisture content in banana pseudostem. The highest dry matter was recorded in Mahalaxmi banana plant 6% and 4.4% in Robusta and Grand Niane. Poyyamozhi and Kadirvel (1986)[19] reported 8.6% dry matter in Robusta banana plant.

**Crude fat and extractive**

The crude fat content was found in the range of 5% - 6.7% (Table 1). The maximum crude fat (6.7%) content was recorded in Grand Naine banana pseudostem followed by Mahalaxmi (5.8%) and Robusta (5.0%). Aziz *et al*., (2011)[8] found very low fat content (0.24%) in *Musa cuminata*x balbisiana Colla. The extractive found in the range of (3.9% - 4.7%). The highest extractive found in Robusta (4.7%) followed by Grand Naine (4.2%) and Mahalaxmi cultivar (3.9%). Similar results were also reported by Rahman *et al*., (2014) [21] for *Musa paradisica*(3.25%). Khan *et al*., (2013)[22] found 3.52% extractive in banana pseudostem.

**Carbohydrate and Protein content**

Carbohydrate content was found in the range of 2.1 - 2.7 mg%. There was no significant difference of carbohydrate content was found in all three different varieties of banana plant. The maximum carbohydrate content was found in Grand Naine (2.7 mg%) followed by Robusta (2.5 mg%) and Mahalaxmi (2.1 mg%). The crude protein content was observed in the range of 80 -190 mg%. The research study shows that the Grand Naine have highest crude protein content (190 mg%). The crude protein content of Mahalaxmi and Robusta were observed to be 80 and 102 mg% respectively. Garg*et al*., (2023)[23] reported 2.8% crude protein content is present in banana pseudostem.

**Mineral analysis**

Banana plant is a rich source of minerals. Table 2 shows the mineral content of three different cultivars of banana pseudostem. The mineral value of banana pseudostem varies with the species of cultivar, stage of maturation and sample preparation and analysis. Sodium, potassium, magnesium, calcium and phosphorous are the macro-elements present in banana pseudostem. Amongst the different minerals, Calcium content was found to be maximum (1484.01, 1357.23 and 1298.67 mg/100 g of dry substrate) in all the three different varieties of banana (Mahalaxmi, Robusta and Grand Naine) respectively. Other macro-elements such as potassium was found in the range of (789.23-869.86 mg/100 g of dry substrate) followed by magnesium (260.51-301.84 mg/100 g of dry substrate), sodium (235.81-265.54 mg/100 g of dry substrate) and phosphorous (198.78-215.84 mg/100 g of dry substrate) in all the three different cultivars of banana. Ho *et al*., (2012)[24] reported similar result for *Musa acuminate* X *balbisiana cv*. They found 1335.33mg/100 g of dry substrate calcium followed by potassium 944.12 mg/100 g of dry substrate, sodium 444.12 mg/100 g of dry substrate, magnesium 255.00 mg/100 g of dry substrate and phosphorous 137.82 mg/100 g of dry substrate. Selema

and Farago (1996)[25] reported highest potassium content 3810 mg/100 g of dry substrate in banana pseudostem of *Musa paradisiaca*.

**Swelling capacity and Solubility of banana pseudostem**

Swelling capacity and solubility of banana pseudostem is depicted in a Table 3. The swelling power is defined as the capacity of the starch present in the dry substrate to absorb the liquid molecules. The swelling capacity and solubility of the substrates depend on the chemical structure, particle size, pH and temperature. The highest swelling power was found in Mahalaxmi banana pseudostem 9.3 g of swollen particles/g of dry substrate and Robusta have lowest swelling capacity 7.3 g of swollen particles/g of dry substrate. Bello-Perez *et al*., (1999)[26] reported that at the higher temperature the solubility enhances the swelling power of the substrate. Aziz *et al*., (2011)[8] obtained 13.82 g swollen particles/g of dry substrate. Robusta have highest solubilized granules 3.6% in comparison to Mahalaxmi and Grand Naine. According to results, there is no significant difference found in the solubilized particle of Mahalaxmi 2.7% and Grand Naine 2.8%.

**Water and Oil absorption capacity**

Water and oil holding capacity defined as the potentiality of one gram of dry substrate to hold or retain water and oil with in its matrix under specific condition. Table 4 shows the water and oil holding capacity of the three banana cultivars. Water absorption capacity of banana pseudostem is found in the range of 10-19 gm of water/g of dry substrate. Grand Naine shows significantly higher water absorption capacity of 19.3 gm of water/g of dry substrate in comparison to Robusta 13.6 gm of water/g of dry substrate and Mahalaxmi 10.7 gm of water/ g of dry substrate. Grand Naine cultivar have high water holding capacity which may be attributed to the high amount of the protein content or the presence of arabinoxylans. Arabinoxyalns are free from the plant cell wall and have good capacity to absorb the water molecules (Choct, 1997)[27]. Aziz *et al*., (2011)[8] reported 10.66 gm of water/g of dry substrate water holding capacity for *Musa acuminate* which is 1.8 time lower than the results obtained in our study for Grand Naine and Robusta.

The oil absorption capacity was found in the range of 4.1- 9.3 gm of oil/g of dry substrate. The maximum oil adsorption capacity was found in the Grand Naine banana pseudostem 9.3 gm of oil/g of dry substrate followed by Robusta and Mahalaxmi 6.4 and 4.1 gm of oil/g of dry substrate respectively. The maximum oil holding capacity is because of the protein content. The presence of protein enhances the oil absorption capacity by entrapping the oil particles[9,28]. Aziz et al., (2011)[8] also found a similar oil holding capacity for the Musa acuminate 5.48 gm of oil/ g of dry substrate.

**Phytochemical analysis**

Alkaloid is a secondary metabolite, which play an important role in human diet. Alkaloids exhibit many important characteristics such as anti-microbial, anti-insecticidal, anti-parasitic, anti-plasmodial and anti-oxidative. Table 5 represents the alkaloid content of three different varieties of banana pseudostem. The research study shows that the maximum amount of alkaloid content was found in Mahalaxmi banana pseudostem (7.8%) followed by Grand Naine (6.6%) and Robusta (6.2%). Onyema*et al*., (2016)[29] reported 8.16% alkaloid content for banana pseudostem of *Musa accuminata.*

The flavonoid content of Mahalaxmi, Robusta and Grand Naine is exhibited in Table 5. The present research analysis shows that the flavonoid content of Mahalaxmi, Robusta and Grand Naine found in the range of 4.7-7.5 µg QE/g of banana pseudostem powder. The maximum flavonoid content was obtained in Mahalaxmi banana pseudostem 7.5 µg QE/g of banana pseudostem powder. The Grand Naine banana pseudostem contains 6.2 µg QE/g flavonoid content. However, the lowest amount of flavonoid was found in Robusta banana pseudostem (4.7 µg QE/g of banana pseudostem powder). Tannin are the class of phytochemicals found in the plant. They are known for their beneficial effects on the metabolism of protein and in enhancing the absorption of the amino acids in the small intestine[30]. The tannin content of three different varieties of banana pseudostem is shown in Table 5. Mahalaxmi banana

pseudostem contains maximum amount of tannin content (12.4%) than the Robusta (10.3%) and Grand Naine (9.5%). Onyema*et al*., (2016)[29] reported 9.13% tannin content for banana pseudostem of *Musa acuminata.*

Table 5 shows the phytate content of the Mahalaxmi, Robusta and Grand Naine banana pseudostem. The result shows that there was no significant difference observed between the three different varieties of banana pseudostem. Mahalaxmi banana pseudostem contains maximum phytate content (37.57 mg/100 g) followed by Robusta (35.75 mg/100 g) and Grand Naine (33.6 mg/100 g). Ramu*et al*., (2017)[31] found similar result for *Musa* sp. CV. Nanjangud Rasa Bale). They reported 34.56 mg/100 g phytate content for banana pseudostem. The phenolic content of three different varieties of banana pseudostem is shown in the Table 5. The total phenolic content of three different varieties of banana pseudostem (Mahalaxmi, Robusta and Grand Naine) is found in the range from 60.43 - 76.57 mg GAE/g. The result shows that amongst the three different cultivars, Mahalaxmi showed higher total phenolic content (76.57 mg GAE/g). Grand Naine showed 65.82 mg GAE/g total phenolic content and Robusta showed very low phenolic content (60.43 mg GAE/g). Ramu*et al*., (2017)[31] reported 188.64 mg/100 g of phenols in banana pseudostem of *Musa* sp. CV. Nanjangud Rasa Bale. Saravanan and Aradhya, (2011)[32] found phenolic content in the range of 7.58 - 291 mg GAE/ g of extract for eight different cultivars.

## CONCLUSION

In the present study, three different cultivars of banana were collected from the local farm. Banana pseudostem is a main lignocellulosic waste of banana plant. The proximate analysis of banana pseudostem varies with the species of banana. Among three different cultivars of banana, Mahalaxmi has the highest cellulosic matter and lowest lignin content. A cellulose rich banana pseudostem fibers can be extracted from Mahalaxmi banana pseudostem and can be utilizing in the making of paper and in preparation of other cellulose derivatives. The banana pseudostem of all the cultivars has high nutritional value which allows their utilization as an animal feed. Banana pseudostem also contains good amount of phytochemical which enhances the medicinal properties.

## DECLARATIONS

**Ethics approval:** Not applicable
**Consent to participate:** Not applicable
**Availability of data and material:** Not applicable
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**Table 1. Compositional analysis of banana pseudostems.**

| | Mahalaxmi | Robusta | Grand Naine |
|---|---|---|---|
| **Cellulose (%)** | 47.6 ± 0.8 | 40.9 ± 1.1 | 42.7 ± 1.2 |
| **Hemicellulose (%)** | 29.4 ± 1.3 | 28.9 ± 0.5 | 23.3 ± 1.9 |
| **Lignin (%)** | 7.7 ± 0.5 | 9.0 ± 0.2 | 8.8 ± 2.3 |
| **Ash (%)** | 4.5 ± 0.5 | 3.5 ± 0.07 | 2.7 ± 0.6 |
| **Extractive (%)** | 3.9 ± 0.2 | 4.7 ± 0.4 | 4.2 ± 0.1 |
| **Crude fat (%)** | 5.8 ± 0.2 | 5.0 ± 0.3 | 6.7 ± 0.2 |
| **Moisture content (%)** | 94.0 ± 0.02 | 95.5 ± 0.03 | 95.6 ± 0.9 |
| **Dry matter (%)** | 6.0 ± 0.02 | 4.4 ± 0.06 | 4.4 ± 0.2 |
| **Carbohydrate (mg%)** | 2.1 ± 0.05 | 2.5 ± 0.1 | 2.7 ± 0.1 |
| **Protein (mg%)** | 80 ± 6.5 | 102 ± 3.9 | 190 ± 8.1 |

All the values shown in the table are the mean of triplicate

**Table 2. Mineral contents of three cultivars of banana pseudostem.**

| Minerals | Mahalaxmi | Robusta | Grand Naine |
|---|---|---|---|
| Sodium | 265.54 ± 6.3 | 258.74 ± 2.7 | 235.81 ± 7.1 |
| Potassium | 869.86 ± 8.40 | 789.23 ± 16.2 | 812.46 ± 1.4 |
| Calcium | 1484.01 ± 5.98 | 1357.23 ± 0.2 | 1298.67 ± 9.2 |
| Magnesium | 260.51 ± 13.50 | 301.84 ± 2.3 | 298.54 ± 0.06 |
| Phosphorous | 198.78 ± 6.77 | 215.84 ± 3.6 | 201.85 ± 7.4 |
| Manganese | 3.1 ±0 0.77 | 2.8 ± 0.14 | 2.1± 0.07 |
| Iron | 7.6 ± 0.56 | 6.8 ± 0.07 | 7.1 ± 0.07 |
| Zinc | 8.2 ± 0.77 | 8.9 ± 0.28 | 7.8 ± 0.1 |

All the values shown in the table are the mean of triplicate
All the values are given mg/100g of dry substrate

**Table 3. Swelling capacity and solubility of three different banana cultivars.**

| Different variety of banana pseudostem | Swelling power (g of swollen particles/ g of dry substrate) | Solubility (%) |
|---|---|---|
| Mahalaxmi | 9.3 ± 0.2 | 2.7 ± 0.1 |
| Robusta | 7.3 ± 0.1 | 3.6 ±0.2 |
| Grand Naine | 8.4 ± 0.2 | 2.8 ± 0.07 |

All the results shown in the table are mean of the triplicate.

**Table 4.Water and Oil holding capacity of banana pseudostems.**

| Different variety of banana pseudostem | WHC (gm of water/ g of dry substrate) | OHC (gm of oil/ g of dry substrate) |
|---|---|---|
| Mahalaxmi | 10.7 ± 0.1 | 4.1 ± 0.1 |
| Robusta | 13.6 ± 0.07 | 6.4 ± 0.1 |
| Grand Naine | 19.3 ± 0.1 | 9.3 ± 0.07 |

All the results shown in the table are mean of the duplicate.
Water holding capacity (WHC) and oil holding capacity (OHC).

**Table 5. Phytochemical analysis of banana pseudostem**

| Phytochemicals | Mahalaxmi | Robusta | Grand Naine |
|---|---|---|---|
| Alkaloid (%) | 7.85 | 6.2 | 6.6 |
| Flavonoids (mg QE/g substrate) | 7.65 | 4.75 | 6.0 |
| Tannin (%) | 12.4 | 10.3 | 9.5 |
| Phytate(mg/100 gm) | 37.57 | 37.42 | 33.6 |
| TPC (mg GAE/g) | 76.57 | 60.43 | 65.82 |

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**RESEARCH ARTICLE**

# Image Normalization with Noise Removal for Early Neurodegenerative Disease Detection

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## ABSTRACT

Automated retinal layer segmentation has become a key technique for the early detection of neurodegenerative diseases such as Alzheimer's and Parkinson's. the dataset was collected from Kaggle repository. This study introduces a novel algorithm, the Bilateral Noise Element Neural Network (BNENN), designed to enhance segmentation accuracy and noise removal in retinal images, Alzheimer's and Parkinson's. The BNENN algorithm integrates bilateral filtering with neural network architecture to effectively suppress noise while preserving critical structural details in retinal images. By utilizing the advanced pattern recognition capabilities of neural networks, the proposed method achieves robust segmentation of retinal layers, even in the presence of significant noise and artifacts. The implementation has done with python. Experimental results on a diverse dataset demonstrate the superior performance of the BNENN algorithm in accurately segmenting retinal layers and removing noise, outperforming Innovative methods in terms of segmentation accuracy and robustness.

**Keywords:** Automated retinal layer segmentation, Alzheimer's, Bilateral Noise Element Neural Network, Neurodegenerative diseases, Parkinson's.

# INTRODUCTION

Front Temporal Dementia (FTD) and other neurodegenerative disorders cause cognitive and behavioral decline over time [1]. After years of asymptomatic progression, during which aberrant proteins gradually build up in the brain, neurodegenerative processes cause function loss [2]. This is followed by these alterations. Intervention with treatments that can delay or prevent the start of these illnesses might be possible if presymptomatic alterations in people could be reliably identified [3]. Nevertheless, it can be difficult to identify a group of presymptomatic people who are certain to get dementia. Researchers often look at those who can develop uncommon autosomal dominant dementias [4]. We can compare carriers and non-carriers at different stages of the illness because half of these people have the mutation. The frontal and temporal lobes, together with the insula, are involved early in all mutations, as the name suggests. Differences can be seen as early as 10 years before the typical age of symptom start [5-6]. On the other hand, other structures, such the thalamus, seem to be involved to varying degrees in the early stages of the illness [7]. Many types of FTD manifest clinically as an imbalance between the afflicted hemispheres, with greater atrophy within the affected hemisphere lending credence to this observation [8]. On the other hand, the afflicted side isn't always the same, and sometimes there isn't even any sign of an imbalance. It is important to consider this imbalance when trying to identify early alterations with any sensitivity [9] since it is likely to begin early in the illness process.

Nerve cells in the substantia nigra degrade in neurodegenerative disorders (NDDs), which affect the brain's ability to produce dopamine [10]. Movement problems are caused by this degeneration, which impacts the synthesis of dopamine [11]. Most people with Parkinson's disease have stiffness, bradykinesia, resting tremor, postural instability, and trouble walking [12]. Characteristic clinical features of HD include chorea, clumsiness, and stumbling [13]. In addition to the usual muscular atrophy, ALS can cause twitches and cramps, which can make walking difficult [14–15]. For all three diseases, the degree to which a person can walk depends on the severity of their illness. Diagnosing NDDs and gauging the degree of illness in an individual patient can be aided by analyzing gait characteristics. For patients to have access to the appropriate therapies, medications, and care, accurate diagnosis of NDDs is the most critical criteria [16–18]. It is also important to tailor motor symptom therapy to the patient's illness severity. Furthermore, it alters their appearance, which can result in incorrect diagnoses and a significant decline in patients' quality of life [19-21]. Disease identification and severity prediction can be assisted by AI-based models that use gait data. Even though there are a few of these models, most of them use one-dimensional gait data that has been analyzed and modified to identify disorders [22–26].
The main contribution of the paper is:

**Image denoising using BNENN**
The following is the organizational scheme for the rest of this work. In Section 2, many writers discuss various methods for detecting neurodegenerative diseases. In Section 3, the GMBF model is shown. Section 4 presents the results of the investigation. Results and future goals are discussed in Section 5, which finishes the section.

**Motivation of the paper**
An important motivation for this research is the critical requirement for reliable automated retinal layer segmentation in the detection of neurodegenerative diseases in their early stages, including Alzheimer's and Parkinson's. A new approach that successfully eliminates noise from retinal images while also greatly improving segmentation accuracy is the Bilateral Noise Element Neural Network (BNENN) method. With its combination of bilateral filtering and sophisticated neural network architecture, BNENN achieves better performance than previous approaches by overcoming noise and artifacts. Showing its potential influence on clinical applications, the end objective is to provide doctors a powerful tool to better early illness identification and monitoring.

**Background study**

Baldacci, F. et al. [1] Numerous subtypes and clinical manifestations were produced by NDD, which were CNS proteinopathies, according to the kind, location, and dynamic spatio-temporal distribution of abnormal proteins. Potential pathophysiological disease biomarkers that might reveal pathogenetic processes at work in the early, asymptomatic phases of illness include the changed, misfolded proteins characteristic of NDD. Chudzik, A. et al. [3] Artificial Intelligence (AI) and digital technologies hold great promise for improving the speed and accuracy of neurodegenerative disease identification. This might pave the way for earlier therapies that halt the illness's development. Research was ongoing to confirm if digital biomarkers and prediction models powered by AI can effectively detect neurodegenerative disorders in their early stages. By analyzing trends in patient data, these technologies have the potential to create more tailored treatment programs.

Cicirelli, G., & D'Orazio, T. [4] in most cases, doctors or highly trained physiotherapists will only watch patients. The goal was to provide medical professionals with a tool that can aid in the diagnosis of neurodegenerative diseases utilizing this kind of automated system, which requires minimal human intervention and greatly facilitates remote analysis. Cury, C. et al. [6] In order to pinpoint potential presymptomatic variations in the thalamic shape, the author used a new approach of statistical shape analysis on a group of people with hereditary FTD. These authors research leads us to believe that variations become apparent five years before to the anticipated start of symptoms. These alterations were also discovered by volumetric analysis and these authors original shape analysis, but these authors technique demonstrated significance that persisted after multiple comparison correction.

Doroszkiewicz, J. et al. [7] the author highlight what was currently known about the most common genetic abnormalities and molecular markers that can be used to diagnose some NDs early on. In this article, the author will go over some of the newly discovered genes, molecular markers, and proteins in the fields of genetics and biochemistry. According to the data presented here, microRNAs (miRNAs) have the potential to be a "perfect biomarker" for neurological diseases. Fazlali, P. et al. [9] The author summarize what was currently known about the most common genetic abnormalities and molecular biomarkers for the early identification of certain NDs in this article. Newly discovered genes, molecular markers, and proteins in the fields of genetics and biochemistry were covered in this article. Based on what the author know about miRNAs from this review, they seem to be a "perfect biomarker" for neurological diseases. Kumar, A. et al. [13] The main reasons for NDs were the molecular alterations and reactions. Most of the studies conducted in this area center on studying genetic mutations, toxic protein accumulation, and neurotoxic chemical synthesis. According to the findings and discussion in this paper, nanotheranostics has great promise for the treatment of several neurodegenerative diseases. Modeling neurodegenerative diseases in animals, cells, and humans using nanotheranostics techniques has changed the game.

Makdissi, S. et al. [15] in the context of NDs, the microbiota-gut-brain axis was gaining more and more recognition. Nevertheless, further investigation was required to clarify the mechanisms by which bacteria interact with the host and to comprehend the interplay of the dietary factors, the microbiota, and the intestinal epithelium.

**Problem definition**

Existing methods such as GAN and **Bilateral Filter** approaches often face challenges in effectively suppressing noise while preserving critical structural details in retinal images. These methods can struggle with robust segmentation accuracy, especially in the presence of significant noise and artifacts, thereby limiting their efficacy in automated retinal layer segmentation for early neurodegenerative disease detection.

## MATERIALS AND METHODS

In this section, we present the proposed method, the Bilateral Noise Element Neural Network (BNENN), designed for automated retinal layer segmentation and noise removal in the context of early neurodegenerative disease detection.

**Dataset collection**

The dataset was collected from Kaggle website https://www.kaggle.com/datasets/tourist55/alzheimers-dataset-4-class-of-images

**Image denoising using bilateral noise element neural network**

One Recent advances in technology for image denoising is the Bilateral Noise Element Neural Network (BNENN). It is especially useful in retinal imaging, which is essential for the early diagnosis of neurodegenerative diseases. This fresh method combines neural network-based pattern recognition with bilateral filtering, which is well-known for its noise reduction and edge preservation capabilities. BNENN stands out when it comes to improving image quality. It does a great job of eliminating noise while keeping important structural features, which leads to very accurate segmentation. In this study, we claim that deterministic hidden units can be transformed into stochastic ones by introducing noise into them. Injecting noise into the training process causes the optimizer and the model to exhibit stochastic behavior. Using well-defined probabilistic formulations, we can assess the typical training technique and offer alternatives for improved optimization by characterizing deterministic hidden units with noise as stochastic hidden units.

$z = g(h_{\emptyset}(x), \in) \sim p_{\emptyset}(z|x)$ ---------- (1)

We use the notation $h_{\emptyset}\,(x)$ to indicate the hidden unit activations which take input x and model parameters $\emptyset$. Combining the activation with noise—which is often derived from a certain probability distribution, such as the Gaussian distribution—creates a noise injection function. This assumption, when coupled with dropout, produces noise in the form of randomly selected hidden units in a layer and a randomly generated variable $z$ representing the hidden layer's activation for a certain sample of dropout.

A neural network training with stochastic hidden units (z) must maximize the marginal likelihood over all of them if
$L_{marginal} = logE_{p_{\emptyset}(z|x)}[P_{\theta}(y|z,x)]$ ---------- (2)

The probability of ground-truth y given input x and hidden units z is denoted as $P_M\ E_{p_{\emptyset}(z|x)}$, where M is the parameter of the output prediction model.

We use the reparameterization approach from for marginalization of noise-constructed stochastic hidden units. The marginalization is carried out over noise, which is provided by, and Eq. (2) is used in lieu of the random variable z.
$L_{marginal} = logE_{p(e)}[p_{\emptyset}(y|g(h_{\emptyset}(x), \in), x)]$ ---------- (3)

where p() is the noise distribution. The optimization of the marginal likelihood over noise is necessary for training a noise-injected neural network, according to Eq. (3).

**Algorithm 1: Bilateral noise element neural network**

- **Input**:
  - Noisy input image ($X_{noisy}$ )
  - Ground truth clean image ($X_{clean}$ ) for supervised learning
  - Parameters for bilateral filtering and neural network architecture
  - Training data (optional)

**Procedure**:

- Apply bilateral filtering to the input image ($X_{noisy}$ ) to reduce noise while preserving edges and structural details.
- Optionally, train the BNENN model using supervised learning with pairs of noisy input images ($X_{noisy}$ ) and corresponding clean images ($X_{clean}$ ).

**Bilateral Filtering**:

- The bilateral filter smooths an image while preserving edges by taking into account both spatial proximity and intensity similarity between pixels.
- The filtered pixel value $I(x)$ at position xx is computed as:

$$I'(x) = \frac{1}{w(x)} \sum_{y \in S} I(y).G_{\sigma_s}(x-y).G_{\sigma_r}(|I(x)-I(y)|)$$

where:

- $I(x)$ and $I(y)$ are the intensity values of pixels at positions $x$ and $y$, respectively.
- $w(x)$is the normalization factor.
- $G_{\sigma_s}$and $G_{\sigma_r}$ are Gaussian kernels for spatial and intensity domains, respectively.
- $S$ is the spatial neighborhood of pixel $x$.

◎ **Output**:

- Denoised output image ($X_{denoised}$ )

## RESULTS AND DISCUSSION

The implementation has done with python, in this section, we explore into the results from the application of the Bilateral Noise Element Neural Network (BNENN) algorithm for automated retinal layer segmentation and noise removal. Figure 2 illustrates the effectiveness of the Bilateral Noise Element Neural Network (BNENN) in enhancing brain images for neurodegenerative disease detection. The left panel shows the original brain input image, which contains noise and artifacts that obscure critical structural details. The right panel displays the same image after denoising with the BNENN algorithm, which integrates bilateral filtering with a neural network to suppress noise while preserving essential details. Figure 3 presents a retinal input image used for automated retinal layer segmentation in the early detection of neurodegenerative diseases. Captured using advanced retinal imaging techniques like optical coherence tomography (OCT), the image reveals multiple distinct retinal layers essential for visual function. Figure 4 presents a spiral input image used in the assessment of Parkinson's disease. This image is derived from a drawing task where individuals are asked to draw or trace a spiral, a test commonly used to evaluate motor function. The spiral pattern reveals critical details about the subject's motor control, such as tremors, irregularities, and deviations, which are characteristic of Parkinson's disease.

The table 2 and figure 5 shows comparing the denoising methods for Alzheimer's Disease (AD) images, three techniques—GAN, Bilateral Filter, and BNENN—were evaluated using PSNR, SSIM, and RMSE metrics. The BNENN method consistently outperformed the other methods. For the image 100.jpg, BNENN achieved a PSNR of 20.64, an SSIM of 0.96, and an RMSE of 0.10, while for 150.jpg, it achieved a PSNR of 20.95, an SSIM of 0.96, and an RMSE of 0.08. In contrast, the Bilateral Filter method scored lower with PSNR values of 18.21 and 19.67, SSIM values of 0.93 and 0.94, and RMSE values of 0.17 and 0.15 for 100.jpg and 150.jpg, respectively. The GAN method performed the least effectively, with PSNR values of 17.04 and 17.64, SSIM values of 0.91 and 0.92, and RMSE values of 0.18 for both images. These results indicate that BNENN provides the highest quality denoising for AD images, achieving the best balance of high PSNR, high SSIM, and low RMSE, thus preserving image quality and structural details most effectively.

The table 3 and figure 6 shows comparison of denoising methods for retinal disease images, three techniques—GAN, Bilateral Filter, and BNENN—were assessed using PSNR, SSIM, and RMSE metrics. The BNENN method demonstrated superior performance across all metrics. For the image 100.jpg, BNENN achieved a PSNR of 16.64, an SSIM of 0.97, and an RMSE of 0.15, while for 150.jpg, it achieved a PSNR of 16.86, an SSIM of 0.98, and an RMSE of 0.14. The Bilateral Filter method showed intermediate results with PSNR values of 14.21 and 15.67, SSIM values of 0.96 and 0.97, and RMSE values of 0.16 and 0.15 for 100.jpg and 150.jpg, respectively. The GAN method yielded the lowest performance, with PSNR values of 13.04 and 14.64, SSIM values of 0.95 for both images, and RMSE values of

0.18 and 0.17. These findings indicate that BNENN is the most effective denoising method for retinal disease images, providing the highest image quality with the best balance of high PSNR, high SSIM, and low RMSE.

The table 4 and figure 7 shows evaluation of denoising methods for Parkinson's disease images, three techniques—GAN, Bilateral Filter, and BNENN—were analyzed using PSNR, SSIM, and RMSE metrics. The BNENN method outperformed the others significantly. For the image 100.jpg, BNENN achieved a PSNR of 14.99, an SSIM of 0.98, and an RMSE of 0.13, while for 150.jpg, it achieved a PSNR of 15.28, an SSIM of 0.98, and an RMSE of 0.12. The Bilateral Filter method showed intermediate performance with PSNR values of 13.92 and 14.01, SSIM values of 0.96 for both images, and RMSE values of 0.14 for both images. The GAN method had the lowest performance, with PSNR values of 12.84 and 13.24, SSIM values of 0.94 and 0.95, and RMSE values of 0.16 and 0.15 for 100.jpg and 150.jpg, respectively. These results indicate that BNENN is the most effective denoising method for Parkinson's disease images, providing superior image quality by achieving the highest PSNR, highest SSIM, and lowest RMSE values, thereby preserving image detail and clarity most effectively.

## CONCLUSION

In Conclusion, the BNENN algorithm is a huge step forward for retinal image processing, especially when it comes to spotting neurodegenerative disorders like Alzheimer's and Parkinson's at an early stage. Retinal layer segmentation is made more accurate and reliable by BNENN, which solves the problem of noise reduction while keeping important structural information intact by combining bilateral filtering with neural network design. The findings of the trial confirm that BNENN outperforms the current state-of-the-art approaches, demonstrating its resilience when faced with images that are noisy or include artifacts. The therapeutic value of BNENN is highlighted by its shown potential in early disease identification and its effective use in properly segmenting retinal layers. The BNENN method outperformed the others significantly. For the image 100.jpg, BNENN achieved a PSNR of 14.99, an SSIM of 0.98, and an RMSE of 0.13, while for 150.jpg, it achieved a PSNR of 15.28, an SSIM of 0.98, and an RMSE of 0.12. With its potential to improve early detection and monitoring of neurodegenerative disorders, this algorithm not only pushes the technical boundary in medical imaging but also offers hope for improved patient outcomes via prompt intervention. Possible avenues for further investigation include honing this method even further, investigating its potential use with additional imaging modalities, and evaluating its efficacy in actual clinical practice.

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**Table 1: Survey of Early Detection Methods for Neurodegenerative Diseases**

| Author | Year | Methodology | Advantage | Limitation |
|---|---|---|---|---|
| Baldacci et al. | 2019 | Early disease detection methods | Protection against neurodegenerative disorders at the forefront | Limited coverage of diverse disease detection methods |
| Candelise et al. | 2020 | Assays for the conversion of protein amyloids in vitro | Improved early diagnosis of neurodegenerative diseases | Reliance on in vitro assays, cannot fully represent in vivo conditions |
| Chudzik et al. | 2024 | Artificial Intelligence and Electronic Biomarkers | Identifying neurodegenerative disorders at an early stage | Dependency on accurate digital biomarker identification |
| Cicirelli & D'Orazio | 2022 | Low-cost video-based system for mobility analysis | Cost-effective neurodegenerative disease detection | Limited scalability and accuracy in complex mobility tests |
| Collin, F. | 2019 | The molecular foundation of ROS | Insight into neurodegenerative disease pathology | Lack of direct applicability to early disease detection |

**Table 2: Denoising value comparison on AD**

| | Denoising value comparison on AD | | | |
|---|---|---|---|---|
| | | PSNR | SSIM | RMSE |
| GAN | 100.jpg | 17.04 | 0.91 | 0.18 |
| | 150.jpg | 17.64 | 0.92 | 0.18 |
| **Bilateral Filter** | 100.jpg | 18.21 | 0.93 | 0.17 |
| | 150.jpg | 19.67 | 0.94 | 0.15 |
| BNENN | 100.jpg | 20.64 | 0.96 | 0.10 |
| | 150.jpg | 20.95 | 0.96 | 0.08 |

**Table 3: Denoising value comparison on retinal disease**

| | Denoising value comparison on retinal disease | | | |
|---|---|---|---|---|
| | | PSNR | SSIM | RMSE |
| GAN | 100.jpg | 13.04 | 0.95 | 0.18 |
| | 150.jpg | 14.64 | 0.95 | 0.17 |
| **Bilateral Filter** | 100.jpg | 14.21 | 0.96 | 0.16 |
| | 150.jpg | 15.67 | 0.97 | 0.15 |
| BNENN | 100.jpg | 16.64 | 0.97 | 0.15 |
| | 150.jpg | 16.86 | 0.98 | 0.14 |

**Table 4: Denoising value comparison on parkinson's disease**

| | Denoising value comparison on parkinson's disease | | | |
|---|---|---|---|---|
| | | PSNR | SSIM | RMSE |
| GAN [24] | 100.jpg | 12.84 | 0.94 | 0.16 |
| | 150.jpg | 13.24 | 0.95 | 0.15 |
| **Bilateral Filter [25]** | 100.jpg | 13.92 | 0.96 | 0.14 |

| | | | | |
|---|---|---|---|---|
| | 150.jpg | 14.01 | 0.96 | 0.14 |
| BNENN | 100.jpg | 14.99 | 0.98 | 0.13 |
| | 150.jpg | 15.28 | 0.98 | 0.12 |

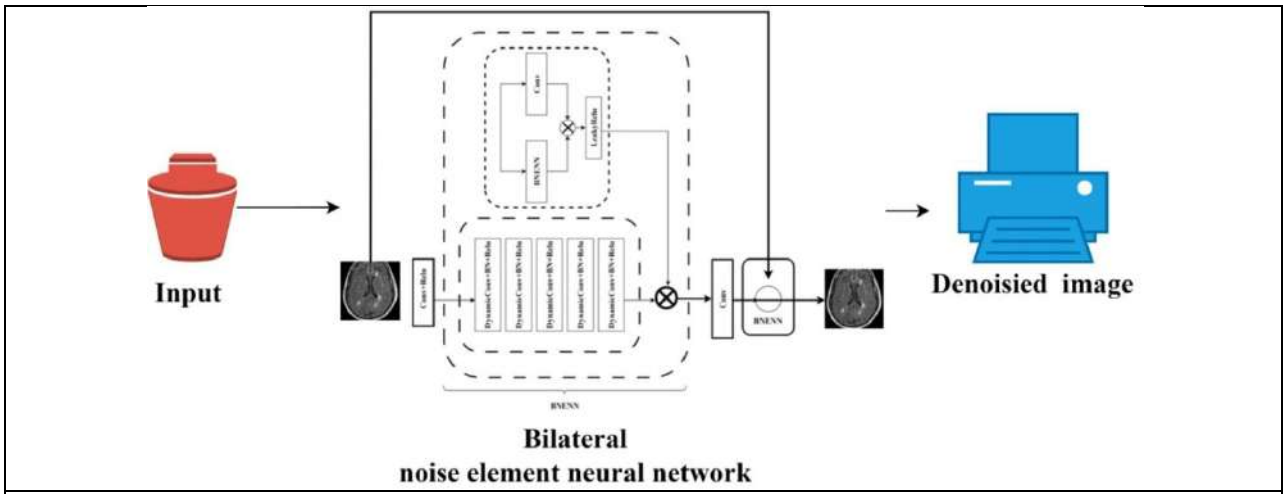


**Figure 1: Proposed workflow architecture**

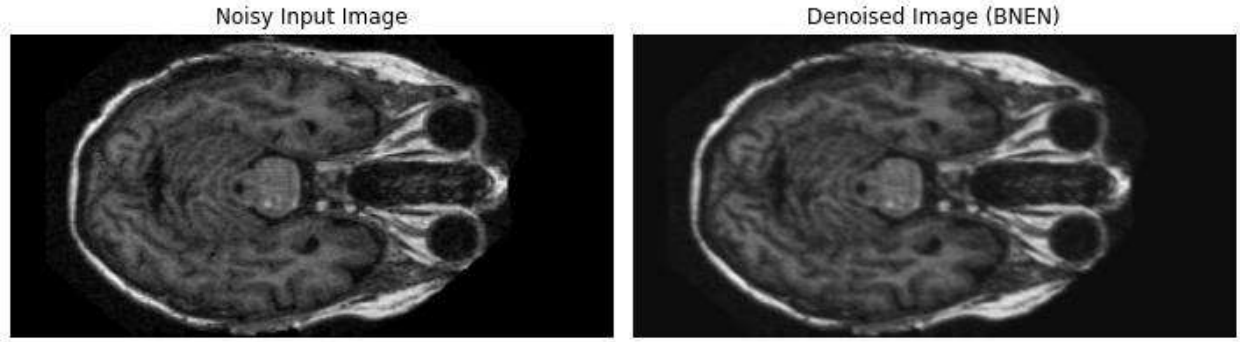


**Figure 2: Brain image**

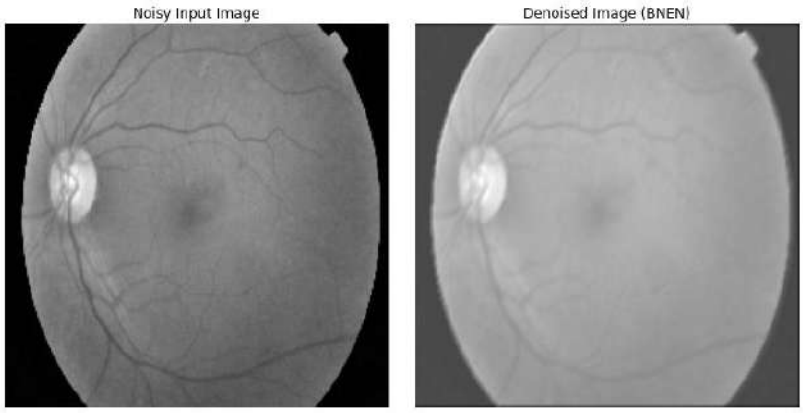


**Figure 3: Retinal image**

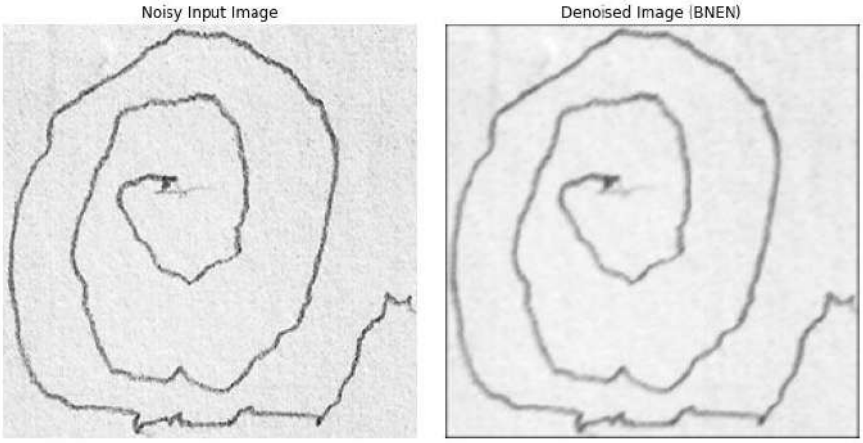


**Figure 4: Spiral image**

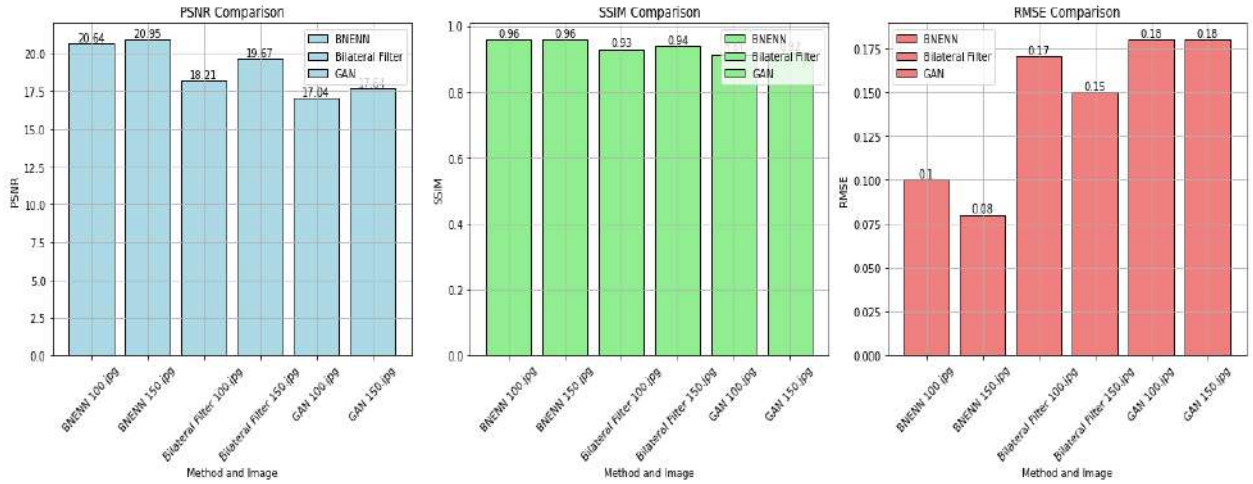


**Figure 5: Denoising value comparison chart for AD**

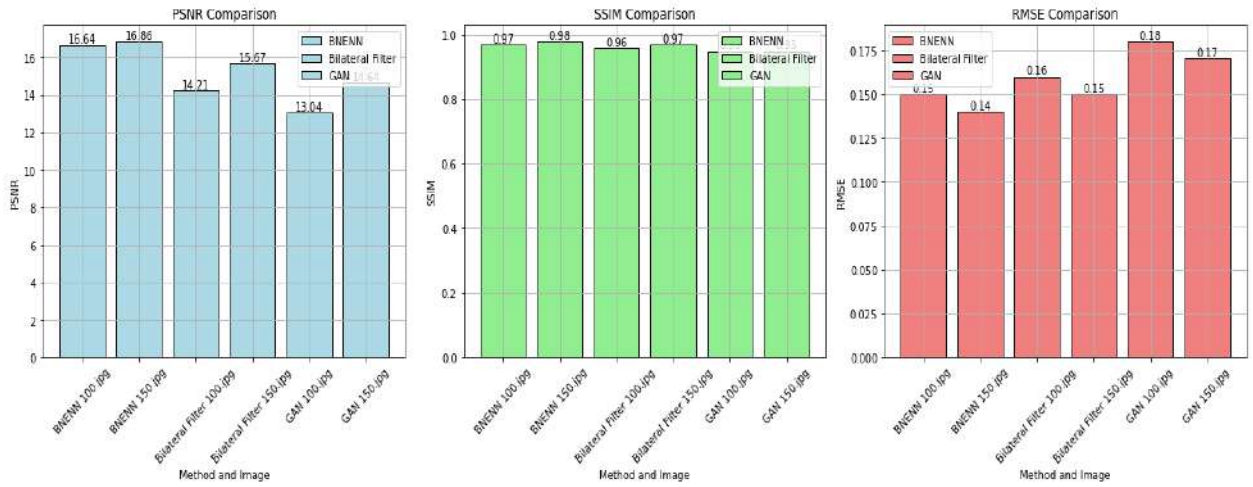


**Figure 6: Denoising value comparison chart for retinal disease**

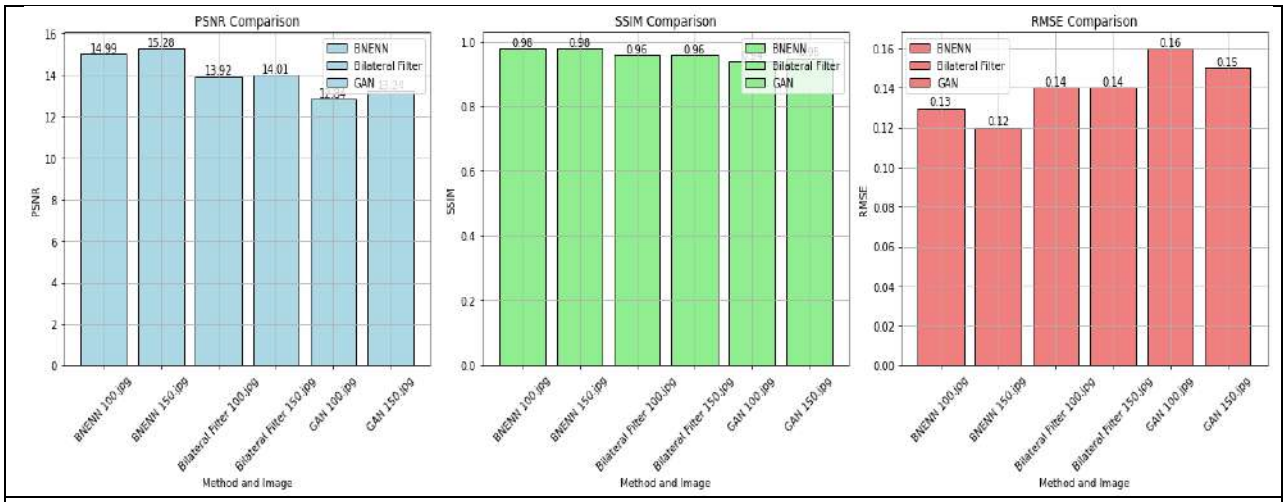


**Figure 7: Denoising value comparison chart for parkinson's disease**

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**RESEARCH ARTICLE**

# Seasonal Variations in Soil Properties Across Altitudinal Gradient of Current Jhum Fields in Champhai District, Mizoram

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## ABSTRACT

Seasonal variations in soil properties were investigated across altitudinal gradients of current jhum fields in Champhai district, Mizoram, India. Soil samples were collected from three locations during the pre-monsoon, monsoon, and postmonsoon seasons (Khawkawn, NE Khawdungsei, and Kawlbem, respectively). The soil texture ranged from loamy sand to sandy loam. The soil moisture content was highest during the monsoon season (21.52 ±0.71% to 24.61 ±1.78%), while the water holding capacity peaked in the postmonsoon period (37.81 ±0.15% to 39.2 ±0.54%). Bulk density was highest during the monsoon season (1.44 ±0.01 to 1.10 ±0.03 g/cm$^3$). The soil pH decreased from the pre-monsoon to postmonsoon. The soil organic carbon content was highest in the pre-monsoon season (1.53 ±0.25% to 2.79 ±0.06%). The available nitrogen (146.35 ±10.45 to 653.33 ±17.02 kg/ha), available phosphorus (20.79 ±0.82 to 34.49 ±0.77 kg/ha), and available potassium (141.59 ±1.97 to 230.82 ±1.54 kg/ha) were highest during the monsoon season. The results emphasise the dynamic nature of the soil and the need for season-specific agricultural strategies for sustainability. They also contribute to the understanding of seasonal soil dynamics in jhum cultivation, a traditional practice central to Mizoram's culture and livelihood, and provide insights for developing techniques to enhance productivity and sustainability in the face of the challenges posed by declining fallow periods and environmental degradation.

**Keywords:** Jhum cultivation, Soil quality, Seasonal variations, Champhai, Mizoram.

## INTRODUCTION

Shifting cultivation, locally known as jhum cultivation, is a predominant agricultural practice in Mizoram, India, where a significant portion of the population (approximately 60%) depends largely on their livelihood [1]. This practice involves clearing forestland followed by burning and cultivating a diverse array of crops, rice, maize, pulses, vegetables, ginger, sesame, cotton, etc, for a period of 1-2 years before abandoning the plot for forest re growth and shifting to a new area [1, 2]. Approximately 54% of Mizoram's arable land is used for jhum agriculture [2], which is generally practiced on steep slopes and is deeply intertwined with Mizo culture, with major festivals linked to the various stages of jhum operations [3].However, the sustainability of jhum cultivation has been compromised owing to a significant reduction in fallow periods, have decreased from 20-25 years [4] to only 3-5 years [1]. This has led to declining yields and increased environmental degradation through soil erosion and watershed siltation [5]. Consequently, the diminishing productivity and sustainability of jhum cultivation in Mizoram's steep terrain necessitates interventions or transitions to more sustainable agricultural practices [6].

Numerous studies have consistently shown the impact of altitude, which significantly influences soil properties, along with other parameters such as texture, pH, and nutrients [7, 8, 9, 10]. Additionally, seasonal variations play a pivotal role in shaping soil quality, influencing microbial activity, abundance, and community structure, which are key indicators of soil health and fertility [11, 12].The Champhai region in Mizoram, India, presents a unique case for studying the impact of season on soil quality within the context of jhum cultivation. This study aimed to evaluate the seasonal variations in soil properties across altitudinal gradients of jhum fields in the study area and discuss their implications for developing sustainable soil management strategies tailored to local conditions. These findings are expected to contribute valuable insights to the body of knowledge on sustainable agricultural practices in hilly and mountainous regions.

## MATERIALS AND METHODS

### Study Site

The study was conducted in Champhai District, Mizoram, which is commonly known as the 'Rice Bowl of Mizoram'. The district spans an area of 3185.83 km² and lies between 93°0'3'' E to 93°26'10'' E and 23°0'33'' N to 24°5'4'' N. According to the 2011 Census, Champhai has a population of 125,745. Despite its significant population, Champhai is the most underdeveloped district in the state, with a substantial number of households relying either directly or indirectly on agricultural activities, primarily jhum cultivation practiced on hill slopes. The district in Mizoram has the highest dependency on this cultivation method at 57.2% [13].

The sampling sites were selected to represent the typical jhum fields in the region, spanning an altitudinal range with varying topography. The selection criteria for the sampling sites included their representation of the commonly practiced jhum cultivation in the district, accessibility, and coverage of the different altitudinal zones. Table 1 and Figure 1 show the locations of the three sampling sites: - Khawkawn (KK), NE Khawdungsei (NE), and Kawlbem (KB) that are spread across different elevation.

The undulating topography of the broken mountain ranges and valleys in Champhai is suitable for crop cultivation, and its extensive forest cover, constituting 83.89% of its geographical area, contributes to its humus-rich soil [14]. A study conducted in this region revealed that the most common jhum field sizes range from 1.0 to 2.0 hectares [14]. The district experiences a moderate climate, with average summer temperatures of 23.5°C (April-June) and average winter temperatures of 15.7°C (November-February), accompanied by heavy rainfall influenced by the southwest monsoon, with an average annual rainfall of 2346.2 mm.

### Soil Sampling and Analysis

Soil samples were taken in replicate from the sampling sites soon after the *jhumias* slash-and-burn activity, covering the pre-monsoon season (February–May), monsoon season (June–September), and postmonsoon season (October–January).In each season, two (2) soil samples from two depths (0-15 cm and 15-30 cm) were collected from each site and stored and air-dried for analysis. Standard techniques were used to analyse the physicochemical properties of the soil samples. These techniques include pH measurements using a digital pH meter, bulk density, soil texture [15], soil moisture content, water-holding capacity [16], soil organic carbon [17] available nitrogen [18], available phosphorus [19], and available potassium [20].

### Statistical analysis

General statistical parameters such as the mean and standard error of mean (SEM), were calculated for each sampling site. Pearson Correlation was used for correlation analysis of the effect of seasonon the soil parameters, and the multiple analysis of variance (ANOVA) and Fisher least significant difference (LSD) tests, aimed at distinguishing between treatment means, were performedat a significance level of 5% ($p \leq 0.05$) using SPSS 27.

## RESULTS AND DISCUSSION

The physical and chemical properties, Spearman's correlation analysis, MANOVA and LSD post-hoc test of the soils across seasons on selected jhum fields were carried out and data were presented and discussed.

### Physical Properties of Soil

The soil texture in the Champhai jhum fields varies from loamy sand to sandy loam in nature. Among the sites, the texture changed from loamy sand in the pre-monsoon and monsoon to sandy loam in the post-monsoon period at Khawkawn (KK).At Khawdungsei (NE), the texture was consistent with loamy sand. At Kawlbem (KB), similar to KK, the texture varied from loamy sand to sandy loam in the post monsoon season. The soil moisture content (SMC) exhibited significant variations across seasons, with the highest values recorded during the monsoon season and the lowest values during the pre-monsoon season. The SMC ranged from 15.15 ± 0.53% to 28.89 ± 0.78% across all sites, depths, and seasons (Table 2).

.The middle altitude (NE) exhibited the highest SMC values during the monsoon season compared with those at other altitudes, and the SMC across all sites and seasons showed that the SMC in the surface layer (0-15 cm) was greater than that in the subsurface (15-30 cm) layer (Table 2).The analysis of variance (ANOVA) results revealed a significant effect of season on SMC ($F = 8.451$, $p = 0.001$) (Table 5).The SMC values during the monsoon season were significantly greater than those during the pre-monsoon (MD = 3.55, $p < 0.001$) and post-monsoon (MD = 4.43, $p < 0.001$) seasons, as per LSD post-hoc test (Table 6).

The WHC values ranged from 30.28 ± 0.03% to 44.71 ± 0.75% across all sites and seasons (Table 2). The WHC showed altitudinal variation as it increased with increasing altitude, with higher altitudes having the highest WHC across seasons and depth. The WHC in the surface layer was generally greater than that in the subsurface layer across all sites and seasons (Table 2).The WHC exhibited a positive correlation with the season ($r = 0.582$, $p < 0.01$) (Table 4).The ANOVA results showed a significant effect of season on the WHC ($F = 16.614$, $p < 0.001$) (Table 5).The post-hoc LSD test further revealed that the WHC values during the pre-monsoon season were significantly lower than those during the monsoon (MD = -4.15, $p < 0.001$) and post-monsoon (MD = -4.88, $p < 0.001$) seasons (Table 6). The bulk density (BD) ranged from 0.89 ± 0.01 g/cm$^3$ to 1.45 ±0.02 g/cm$^3$ across all sites, depths and seasons (Table 2). BD decreased with increasing altitude across depth and season, while BD in the subsurface layer was greater than that in the surface layer across all sites and seasons. The ANOVA did not show a significant effect of season on BD ($F = 1.461$, $p = 0.242$) (Table 5).

**Chemical Properties of Soil**

The soil pH ranged from 5.48 ±0.03 to 6.21 ±0.02 across all sites and seasons (Table 3).The pH exhibited altitudinal variation, generally decreasing from lower to middle altitudes, but increasing from middle to higher altitudes across seasons and depths. The soil pH in the surface layer was greater than that in the subsurface layer across all sites and seasons (Table 3).ANOVA revealed a significant effect of season on soil pH ($F = 24.950$, $p < 0.001$) (Table 5).The post-hoc LSD test further revealed that the soil pH values during the pre-monsoon season were significantly greater than those during the monsoon (MD = 0.37, $p < 0.001$) and post-monsoon (MD = 0.16, $p < 0.001$) seasons, and that the soil pH during the post-monsoon season was significantly higher than that during the monsoon season (MD = 0.21, $p < 0.001$) (Table 6).

The soil organic carbon (SOC) content of the soils ranged from 0.76 ± 0.10% to 2.92 ± 0.04% across all sites, depths, and seasons (Table 3). SOC showed altitudinal variation, as the SOC content in the middle latitudes showed greater across all seasons and depths than in the lower and higher altitudes, while SOC content in the surface layer was greater than that in the subsurface layer across all sites and seasons (Table 3). Although the ANOVA did not reveal a significant effect of season on SOC ($F = 1.789$, $p = 0.177$) (Table 5), SOC exhibited a general decreasing trend with increasing soil depth, which is consistent with the typical distribution of organic matter in soil profiles.

The available nitrogen (AN) content in the soils exhibited significant seasonal variation, with the highest values observed during the monsoon season and the lowest during the pre-monsoon season (Table 3). The AN ranged from 135.89 ±10.45 kg/ha to 669.01 ±20.91 kg/ha across all sites and seasons and the AN showed altitudinal variation as it increased with increasing altitude (Table 3), across all seasons and sites, the surface layer had greater AN content than did the subsurface layer (Table 3). ANOVA revealed a significant effect of season on AN ($F = 19.391$, $p < 0.001$) (Table 5), and the post-hoc LSD test further confirmed that AN levels during the monsoon season were significantly higher than those during both the pre-monsoon (MD = 213.87, $p < 0.001$) and post-monsoon (MD = 194.71, $p < 0.001$) seasons (Table 6).

The available phosphorus (AP) content in the soils exhibited a distinct seasonal pattern, with higher values observed during the monsoon season than the pre-monsoon and post-monsoon seasons (Table 3). The AP ranged from 18.10 ±0.48 kg/ha to 35.75 ±1.16 kg/ha across all sites, depths and seasons (Table 3). The AP showed altitudinal variation as it increased with increasing altitude, and the AP content in the surface layer was greater than in the subsurface layer across altitudes and seasons (Table 3).ANOVA revealed a significant effect of season on AP ($F = 27.154$, $p < 0.001$) (Table 5).The AP during the monsoon season was significantly greater than that during both the pre-monsoon (MD = 8.65, $p < 0.001$) and post-monsoon (MD = 4.56, $p < 0.001$) seasons, as per the LSD post-hoc test (Table 6).

The available potassium (AK) content in the soils exhibited seasonal variation, with higher values observed during the monsoon season than during the pre-monsoon and post-monsoon seasons (Table 3). The AK values ranged from 138.28 ±1.16 kg/ha to 248.14 ±3.39 kg/ha across all sites, depths and seasons (Table 3), and the AK exhibited greater altitudinal variation at middle altitudes than at higher and lower altitudes across all seasons and depths (Table 3). The AK also varied with in depth, as the AK content in the surface layer was greater than that in the subsurface layer across sites and seasons (Table 3).ANOVA revealed a significant seasonal effect on AK ($F = 6.117$, $p = 0.004$) (Table 5).The post-hoc LSD test further confirmed that AK levels during the monsoon season were significantly greater than those during the pre-(MD = 34.38, $p < 0.001$) and post-monsoon (MD = 16.39, $p < 0.001$) seasons (Table 6).

The observed seasonal patterns in soil properties were consistent with findings from tropical and subtropical regions [21, 22, 23, 24], which exhibit greater nutrient availability, particularly nitrogen and phosphorus, during the monsoon season due to increased microbial activity and mineralisation processes facilitated by favourable soil moisture conditions [25, 26, 27, 28, 29, 30]. The shifts in soil texture, with higher sand content during the monsoon season, can be attributed to the erosive effects of heavy rainfall selectively removing finer particles, such as silt and clay, leaving behind coarser sand fractions, as observed in other tropical regions with distinct dry and wet seasons [31].

The higher SMC and WHC during the monsoon and post-monsoon seasons, respectively, align with studies in which soil moisture dynamics are influenced by seasonal rainfall patterns [32, 33, 34]. A study in the lower Siwalik foothills of the Himalayas reported higher soil SOC for different land use systems during the pre-monsoon season [35].

The observed seasonal patterns in soil properties are not limited to the jhum cultivation system but have also been reported across various land use systems, including conventional agriculture, agro forestry, and natural ecosystems.[36] conducted a study in the lower Shivalik foothills of Punjab, India, and reported significant seasonal variations in the physical and chemical properties of soil under different land-use systems, including farm forestry and horticulture systems. They observed a higher soil moisture content and nutrient availability during the monsoon season, which is consistent with the findings of the present study. A study conducted by [37] in the Brazilian Amazon also revealed significant seasonal changes in soil moisture, nutrient availability, and microbial activity across different land-use systems, including native forests, agro forestry systems, and pastures. These seasonal variations were primarily driven by contrasting rainfall patterns and temperature regimes between the dry and wet seasons, similar to the observations made in the present study.

It is important to note that the observed seasonal variations in soil properties have implications for soil fertility management and agricultural productivity [23]. investigated the influence of seasonal fluctuations on soil characteristics and microbial populations in tropical dry deciduous forests and reported that the SMC dramatically increases in the summer, thereby increasing the amounts of SOC and macro- and micronutrients [38], reported that land-use type, soil depth, and season significantly influenced microbial activity, biomass of different soil layers, soil properties, and microbial biomass carbon, all of which exhibited strong seasonality, highlighting the need for season-specific nutrient management strategies to optimise crop yields and maintain soil fertility.

Although the current study provides insightful information about the dynamics of soil properties in the Champhai, Mizoram's jhum cultivation systems, it is important to recognise its potential limitations, including the absence of long-term monitoring data and the impact of variables, such as vegetation cover and management techniques, on soil properties. Future research should consider incorporating long-term monitoring of soil properties across multiple growing seasons and evaluating the interactive effects of land use practices and seasonal variations on soil quality and productivity.

## CONCLUSION

This study revealed significant seasonal variations in key soil properties, including moisture, nutrient content, and pH, across altitudinal gradients. These findings demonstrate the dynamic nature of soil and the necessity of season-specific soil management strategies to improve soil productivity and sustainability. Although this study provides valuable insights, it is important to acknowledge its limitations, such as the lack of long-term data and the potential influences of vegetation and management practices. To address these limitations, future research should incorporate multiyear monitoring programs and investigate the interactive effects of various factors on soil quality. This study emphasises the need for a comprehensive soil-monitoring program that considers seasonal and topographic variations in soil properties. Such a program could inform policymaking and the development of tailored soil management methods for jhum farming, while supporting environmental conservation and cultural preservation. In conclusion, this study highlights the importance of understanding soil dynamics and adapting agricultural practices to local conditions. Integrating scientific knowledge with traditional wisdom may help balance jhum productivity with long-term sustainability amid challenges such as the decline in fallow periods.

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**Table 1. Location of study sites**

| Elevation (asl) | Sites | Coordinates | Altitude (m) |
|---|---|---|---|
| Lower (<500 m) | Khawkawn (KK) | 24°01'24"N | 344 |
| | | 93°15'10"E | |
| Middle (500-1000 m) | NE Khawdungsei (NE) | 23º57'22"N | 829 |
| | | 93º13'31"E | |
| Higher (>1000 m) | Kawlbem (KB) | 23°53'16"N | 1579 |
| | | 93°16'36"E | |

**Table 2. Physical properties of soil of current jhum fields in Champhai, Mizoram (Mean ±SEM)**

| Sites/Altitude | Seasons | Depth | Texture | SMC (%) | WHC (%) | BD (g/cm3) |
|---|---|---|---|---|---|---|
| KK (344) | Pre-monsoon | 0-15 | Loamy sand | 17.75±0.8 | 31.91±0.03 | 1.32±0.06 |
| | | 15-30 | | 15.15±0.53 | 30.28±0.03 | 1.41±0.06 |
| | Monsoon | 0-15 | Loamy sand | 22.97±0.54 | 35.93±0.06 | 1.42±0.01 |
| | | 15-30 | | 20.07±0.35 | 33.45±0.37 | 1.45±0.02 |
| | Post monsoon | 0-15 | Sandy loam | 18.81±0.30 | 38.13±0.06 | 1.39±0.02 |
| | | 15-30 | | 17.64±0.47 | 37.48±0.03 | 1.43±0.01 |
| NE (829) | Pre-monsoon | 0-15 | Loamy sand | 24.51±0.91 | 36.27±0.01 | 1.13±0.04 |
| | | 15-30 | | 22.79±0.72 | 32.49±0.03 | 1.15±0.03 |
| | Monsoon | 0-15 | Loamy sand | 28.89±0.78 | 39.11±0.23 | 1.19±0.02 |
| | | 15-30 | | 23.08±0.66 | 37.99±0.20 | 1.21±0.02 |
| | Post monsoon | 0-15 | Loamy sand | 20.23±0.73 | 40.14±0.04 | 1.16±0.02 |
| | | 15-30 | | 18.88±0.05 | 39.41±0.06 | 1.17±0.02 |
| KB (1579) | Pre-monsoon | 0-15 | Loamy sand | 24.51±1.03 | 38.02±1.04 | 0.89±0.01 |
| | | 15-30 | | 18.22±0.78 | 35.3±0.99 | 0.98±0.02 |
| | Monsoon | 0-15 | Loamy sand | 28.54±0.50 | 44.71±0.75 | 1.05±0.04 |
| | | 15-30 | | 20.68±0.33 | 38±0.13 | 1.15±0.01 |
| | Post monsoon | 0-15 | Sandy loam | 24.21±1.27 | 40.38±0.27 | 0.93±0.02 |
| | | 15-30 | | 17.9±0.55 | 38.02±0.10 | 1.01±0.01 |

**Table 3. Chemical properties of soils in current jhum fields of Champhai, Mizoram (Mean ±SEM)**

| Site/Altitude | Seasons | Depth | pH | OC (%) | AN (kg/ha) | AP (kg/ha) | AK (kg/ha) |
|---|---|---|---|---|---|---|---|
| KK (344) | Pre-monsoon | 0-15 | 5.8±0.04 | 2.08±0.07 | 156.8±18.11 | 21.8±1.11 | 144.9±2.68 |
| | | 15-30 | 5.76±0.04 | 0.98±0.07 | 135.89±10.45 | 19.77±1.06 | 138.28±1.16 |
| | Monsoon | 0-15 | 5.6±0.04 | 1.89±0.09 | 449.49±10.45 | 33.61±1.02 | 214.25±2.55 |
| | | 15-30 | 5.54±0.03 | 0.76±0.10 | 321.51±19.76 | 25.46±0.82 | 211.57±1.77 |
| | Post monsoon | 0-15 | 5.63±0.04 | 1.96±0.06 | 240.43±27.66 | 26.61±0.42 | 182.17±2.01 |
| | | 15-30 | 5.59±0.01 | 0.85±0.08 | 229.97±20.91 | 21.78±0.70 | 175.9±1.89 |
| NE(829) | Pre-monsoon | 0-15 | 6.21±0.02 | 2.88±0.20 | 397.23±37.69 | 19.85±0.76 | 239.57±1.9 |
| | | 15-30 | 5.85±0.03 | 2.12±0.04 | 344.96±18.11 | 18.1±0.48 | 232.03±2.12 |
| | Monsoon | 0-15 | 5.57±0.02 | 2.29±0.10 | 501.76±18.11 | 27.15±1.51 | 248.14±3.39 |
| | | 15-30 | 5.48±0.03 | 1.69±0.03 | 428.59±10.45 | 25.02±0.62 | 243.76±3.32 |
| | Post monsoon | 0-15 | 5.97±0.07 | 2.66±0.06 | 376.32±18.11 | 24.72±0.41 | 242.41±2.42 |
| | | 15-30 | 5.66±0.03 | 1.25±0.03 | 219.52±36.21 | 23.06±0.23 | 235.65±2.22 |
| KB (1579) | Pre-monsoon | 0-15 | 6.18±0.01 | 2.92±0.04 | 439.04±18.11 | 25.31±1.07 | 216.49±2.26 |
| | | 15-30 | 5.84±0.01 | 2.66±0.02 | 250.88±18.11 | 23.47±0.80 | 201.83±1.35 |
| | Monsoon | 0-15 | 5.65±0.04 | 2.31±0.03 | 669.01±20.91 | 35.75±1.16 | 233.09±2.42 |
| | | 15-30 | 5.59±0.02 | 2.26±0.26 | 637.65±27.66 | 33.23±0.20 | 228.56±0.95 |
| | Post monsoon | 0-15 | 5.97±0.09 | 2.59±0.29 | 407.64±18.14 | 29.29±1.05 | 225.54±1.92 |
| | | 15-30 | 5.85±0.05 | 2.44±0.19 | 365.87±27.66 | 27.42±0.38 | 219.38±2.17 |

**Table 4. Correlations between seasons and soil parameters of current jhum fields in Champhai, Mizoram**

| | Seasons | pH | SMC | WHC | BD | OC | AN | AP | AK |
|---|---|---|---|---|---|---|---|---|---|
| Seasons | 1 | | | | | | | | |
| pH | -.303* | 1 | | | | | | | |
| SMC (%) | -0.093 | 0.101 | 1 | | | | | | |
| WHC (%) | .582** | -0.026 | .539** | 1 | | | | | |
| BD (g/cm3) | 0.081 | -.555** | -.385** | -.440** | 1 | | | | |
| OC (%) | -0.189 | .643** | .420** | .344* | -.727** | 1 | | | |
| AN (kg/ha) | 0.053 | -0.043 | .735** | .609** | -.389** | .416** | 1 | | |
| AP (kg/ha) | .340* | -.290* | .446** | .614** | -0.161 | 0.188 | .712** | 1 | |
| AK (kg/ha) | 0.23 | 0.08 | .668** | .606** | -.474** | .413** | .681** | .320* | 1 |

**Table 5. Multiple analyses of variance (ANOVA) between soil parameters and seasons**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| pH | Between Groups | 1.225 | 2 | 0.612 | 24.95 | 0 |
| | Within Groups | 1.252 | 51 | 0.025 | | |
| | Total | 2.477 | 53 | | | |
| SMC (%) | Between Groups | 197.745 | 2 | 98.873 | 8.451 | 0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | 596.702 | 51 | 11.7 | | |
| | Total | 794.447 | 53 | | | |
| WHC (%) | Between Groups | 249.639 | 2 | 124.819 | 16.614 | 0 |
| | Within Groups | 383.167 | 51 | 7.513 | | |
| | Total | 632.805 | 53 | | | |
| BD (g/cm$^3$) | Between Groups | 0.094 | 2 | 0.047 | 1.461 | 0.242 |
| | Within Groups | 1.634 | 51 | 0.032 | | |
| | Total | 1.728 | 53 | | | |
| OC (%) | Between Groups | 1.653 | 2 | 0.826 | 1.789 | 0.177 |
| | Within Groups | 23.55 | 51 | 0.462 | | |
| | Total | 25.203 | 53 | | | |
| AN (kg/ha) | Between Groups | 504116.033 | 2 | 252058.017 | 19.391 | 0 |
| | Within Groups | 662929.998 | 51 | 12998.627 | | |
| | Total | 1167046.031 | 53 | | | |
| AP (kg/ha) | Between Groups | 674.547 | 2 | 337.274 | 27.154 | 0 |
| | Within Groups | 633.461 | 51 | 12.421 | | |
| | Total | 1308.008 | 53 | | | |
| AK (kg/ha) | Between Groups | 10644.866 | 2 | 5322.433 | 6.117 | 0.004 |
| | Within Groups | 44375.076 | 51 | 870.1 | | |
| | Total | 55019.942 | 53 | | | |

**Table 6. LSD post-hoc test between soil parameters and seasons of current jhum fields in Champhai, Mizoram**

| | Dependent Variable | | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| pH | Pre-monsoon | Monsoon | .3678* | 0.04 | 0 | 0.2831 | 0.4524 |
| | | Post monsoon | .1589* | 0.04 | 0 | 0.0742 | 0.2436 |
| | Monsoon | Pre-monsoon | -.3678* | 0.04 | 0 | -0.4524 | -0.2831 |
| | | Post monsoon | -.2089* | 0.04 | 0 | -0.2936 | -0.1242 |
| | Post monsoon | Pre-monsoon | -.1589* | 0.04 | 0 | -0.2436 | -0.0742 |
| | | Monsoon | .2089* | 0.04 | 0 | 0.1242 | 0.2936 |
| SMC (%) | Pre-monsoon | Monsoon | -3.5494* | 0.93 | 0 | -5.4156 | -1.6833 |
| | | Post monsoon | 0.8767 | 0.93 | 0.349 | -0.9895 | 2.7428 |
| | Monsoon | Pre-monsoon | 3.5494* | 0.93 | 0 | 1.6833 | 5.4156 |
| | | Post monsoon | 4.4261* | 0.93 | 0 | 2.56 | 6.2922 |
| | Post monsoon | Pre-monsoon | -0.8767 | 0.93 | 0.349 | -2.7428 | 0.9895 |
| | | Monsoon | -4.4261* | 0.93 | 0 | -6.2922 | -2.56 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| WHC (%) | Pre-monsoon | Monsoon | -4.1544* | 0.59 | 0 | -5.3498 | -2.9591 |
| | | Post monsoon | -4.8806* | 0.59 | 0 | -6.0759 | -3.6852 |
| | Monsoon | Pre-monsoon | 4.1544* | 0.59 | 0 | 2.9591 | 5.3498 |
| | | Post monsoon | -0.7261 | 0.59 | 0.228 | -1.9215 | 0.4692 |
| | Post monsoon | Pre-monsoon | 4.8806* | 0.59 | 0 | 3.6852 | 6.0759 |
| | | Monsoon | 0.7261 | 0.59 | 0.228 | -0.4692 | 1.9215 |
| AN (kg/ha) | Pre-monsoon | Monsoon | -213.8689* | 21.38 | 0 | -256.9257 | -170.8121 |
| | | Post monsoon | -19.1578 | 21.38 | 0.375 | -62.2146 | 23.8991 |
| | Monsoon | Pre-monsoon | 213.8689* | 21.38 | 0 | 170.8121 | 256.9257 |
| | | Post monsoon | 194.7111* | 21.38 | 0 | 151.6543 | 237.7679 |
| | Post monsoon | Pre-monsoon | 19.1578 | 21.38 | 0.375 | -23.8991 | 62.2146 |
| | | Monsoon | -194.7111* | 21.38 | 0 | -237.7679 | -151.6543 |
| AP (kg/ha) | Pre-monsoon | Monsoon | -8.6533* | 0.79 | 0 | -10.2462 | -7.0605 |
| | | Post monsoon | -4.0983* | 0.79 | 0 | -5.6912 | -2.5055 |
| | Monsoon | Pre-monsoon | 8.6533* | 0.79 | 0 | 7.0605 | 10.2462 |
| | | Post monsoon | 4.5550* | 0.79 | 0 | 2.9622 | 6.1478 |
| | Post monsoon | Pre-monsoon | 4.0983* | 0.79 | 0 | 2.5055 | 5.6912 |
| | | Monsoon | -4.5550* | 0.79 | 0 | -6.1478 | -2.9622 |
| AK (kg/ha) | Pre-monsoon | Monsoon | -34.3790* | 1.77 | 0 | -37.9361 | -30.8219 |
| | | Post monsoon | -17.9867* | 1.77 | 0 | -21.5438 | -14.4295 |
| | Monsoon | Pre-monsoon | 34.3790* | 1.77 | 0 | 30.8219 | 37.9361 |
| | | Post monsoon | 16.3923* | 1.77 | 0 | 12.8352 | 19.9495 |
| | Post monsoon | Pre-monsoon | 17.9867* | 1.77 | 0 | 14.4295 | 21.5438 |
| | | Monsoon | -16.3923* | 1.77 | 0 | -19.9495 | -12.8352 |

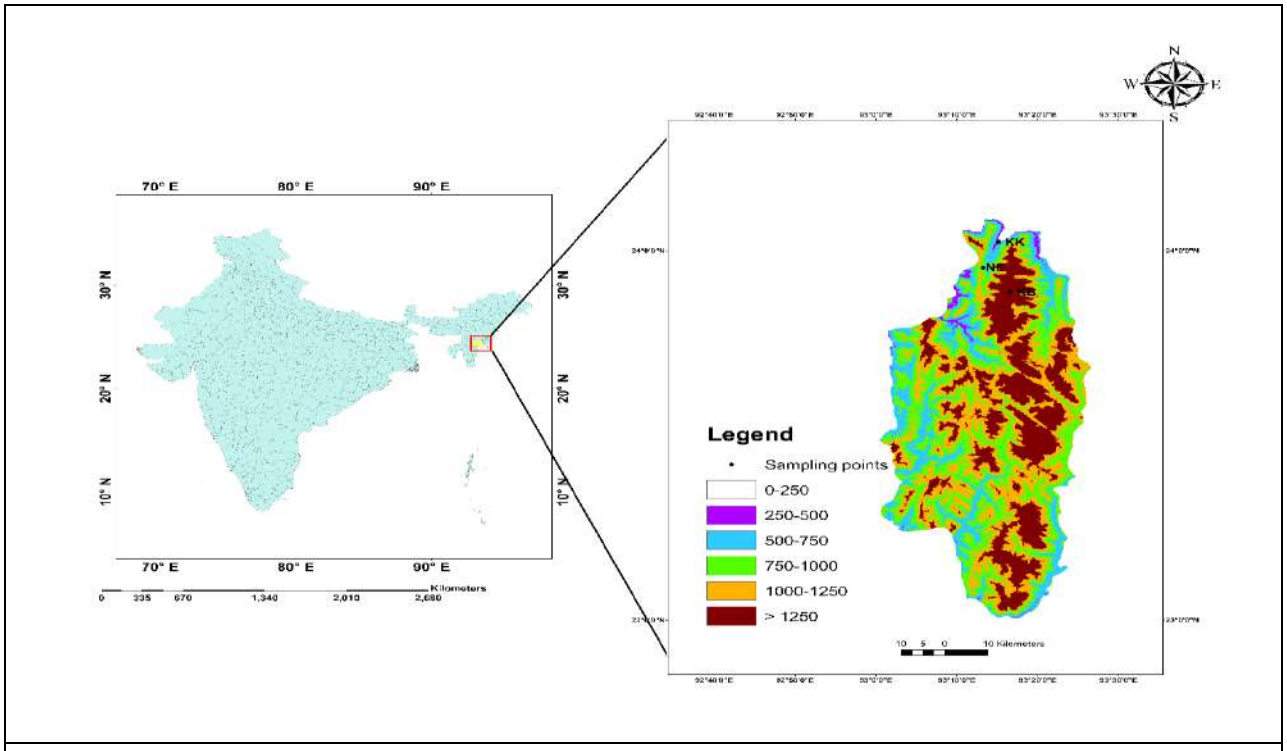


**Fig. 1. Location of study site and demographic elevation map (DEM) of Champhai showing sampling sites**

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**RESEARCH ARTICLE**

# Variation in Water Structures from One Organ to Another in a Goat and Their Response to Homeopathic Potencies Derived from Natural Substances : A Spectroscopic Analysis

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## ABSTRACT

Homeopathic potencies are specifically structured water which can modify the water structure of ordinary water. Water in the body of a living organism is structured because of the presence of biomolecules and cell membranes. Are the water structures same in different tissues of the body? The present study addresses this question. The study further explores the interaction between the water structure in a tissue and a related homeopathic potency. Water structures of five different organs like brain, kidney, lungs, liver and stomach were studied by electronic and vibrational spectroscopy. The response of water structure of organ samples to related homeopathic potencies like *Hypericum perforatum* 200 (Plant product), *Mercurius corosivus* 30 (Salt), *Phosphorus* 30 (Natural element), *Chelidonium majus*200 (Plant) and *Cina* 30 cH (Plant) was also studied by the same spectroscopic methods. Water structures in the five organs tested are different from each other. The responses of these water structures to five related homeopathic potencies are also different from each other. An appropriate homeopathic potency may selectively modify the water structure of the concerned organ affected. In this way the potency may initiate possible therapeutic effect on the affected organ / tissue.

**Keywords:** Mammalian organs, Water structures, Responses, Homeopathic potency, UV-spectra, FT-IR-spectra.

## INTRODUCTION

Using electronic and vibrational spectroscopy it was demonstrated that different homeopathic potencies carry specific water structures [1-2]. Water structures in a potency is transferable to aqueous ethanol (EtOH) solution [3]. Living tissues carry structured water because of the presence of biomolecules suspended in water, and lipid bilayer in the plasma membrane [4-6]. Are the water structures same in different tissues of a mammal? The present experimental study aims to address the question. The second objective is to see whether the structured water in different tissues responds to a related homeopathic potency. We collected five organ samples of a freshly decapitated castrated goat from a local meat shop. The cut pieces of the organ samples were immediately kept in 90% ethanol so that aqueous ethanol can acquire the water structure of the organ and preserve it. The water structures in the five organ samples were studied by electronic and vibrational spectroscopy. Further, the responses of the water structures of the five organ samples to related homeopathic potencies were also studied [7]. For instance, response of the brain sample to *Hypericum perforatum* 200 cH (derived from plant), kidney sample to *Mercurius corosivus* 30 (derived from salt), lungs sample to *Phosphorus* 30 (natural element), liver sample to *Chelidonium majus*200 (Plant) and stomach sample to *Cina* 30 cH (plant). *Cina* was selected because all goats including the present one understudy are naturally infected by flat worm parasites in their stomach. The spectroscopic characteristics of the water structures in the organ samples have been found to be different. The responses of these water structures to the related homeopathic potencies also show difference in their spectroscopic characteristics.

## MATERIALS AND METHODS

**Collection of organ samples**

Samples of five different organs like brain, lungs, stomach, liver and kidney were collected from a freshly decapitated castrated goat weighing 17 kilograms from a local meat shop. The samples, one gram each, were immediately kept in 5 ml of 90% EtOH so that the solvent can acquire and preserve the water structure of the organ.

**Drugs**

Homeopathic potencies were purchased from a local market in sealed vials. All drugs are in 90% EtOH. The control consists of blank 90% EtOH prepared from absolute ethanol (HIMEDIA,Lot number 0000498722).The drugs are *Hypericum perforatum* 200 (HapcoHahnemann Publishing Company Private LTD. Batch number 8432), *Mercurius corosivus* 30 (Hapco Hahnemann Publishing Company Private LTD. Batch number 7965), *Phosphorus* 30 (King & Co., M. Bhattacharyya & Co. Pvt. Ltd. Batch number D2080), *Chelidonium majus*200 (Hapco Hahnemann Publishing Company Private LTD. Batch number 7955) and *Cina* 30 (Sett Dey & Co (Homoeo) Lab, Batch number 13796). The drugs were selected on the basis of their possible relation to the organ samples tested. Thus *Hypericum* was selected for brain, *Merc cor* for kidney, *Phosphorus* for lungs, Chelidonium for liver and *Cina* for stomach [7]. *Cina* was selected because all goats are infected in their stomach by flat worm parasites.

**Electronic Spectroscopy (UV)**

All organ samples in 90% EtOH were first filtered through Whatman number I filter paper. The filtrate was diluted in deionized and distilled water (DD) 1:100. A pair of quartz cuvettes filled with DD water, and baseline was set in a UV-VIS spectrophotometer (Shimadzu, Model- UV-VIS 1900i, Software-Lab Solutions UV-VIS) at room temperature 24±2 ℃,with the instrument in spectrum mode in the wavelength range 190-210nm, scan speed medium and data interval 0.5 nm). Then one cuvette was taken out, emptied washed in DD water and filled with the filtrate of organ samples, and the spectrum was recorded in the wavelength range of 200 nm to 300 nm. In another experiment we tested the response of each filtrate of an organ to a related homeopathic potency. Each potency was diluted with DD water 1:100 and then mixed with a filtrate from an organ in the proportion 1:1. The control consisted of diluted EtOH mixed with a diluted potency in the proportion 1:1. As for example, the brain sample was treated with *Hypericum*

200. The control consisted of diluted EtOH + *Hypericum* 200. In this way all the five organ samples were treated with a related homeopathic potency and their UV-spectra were recorded in the wavelength range 200 nm to 300 nm.

**FT-IR spectra**

FT-IR spectra of all the five organ samples were recorded with the help of a Shimadzu IR Affinity -1S Fourier Transform Infrared (FT-IR) spectrophotometer (Spectrum two) using the attenuated total reflection (ATR) technique. The energy resolution was 0.5 $cm^{-1}$. The baseline was corrected for atmospheric humidity and $CO_2$. One drop of each sample was put into the sample groove, and then the tip of a single reflection pure diamond crystal was brought in contact with the sample drop. The entire spectrum was recorded in the wave number range of 4000 to 500 $cm^{-1}$. Each spectrum represents an average of 45 scans. The temperature and humidity were maintained in the laboratory at $24^0$C and less than 50%, respectively. FT-IR spectra of all the five drugs treated with related homeopathic potencies as mentioned under UV spectra were also recorded.

## RESULTS

**UV spectra**

All the spectra show distinct peak at 200 nm. The highest intensity is found in the brain sample followed by kidney, stomach, liver and lungs. The brain and the kidney showed one more broad peak covering the region between 240 nm and 280 nm (fig.1). All the treatment effects of organ samples + related homeopathic potency and their controls showed single peak at 200 nm. In all the cases the organ samples + related homeopathic potency showed higher intensity then their control (fig. 2 a, b, c, d and e).

**FT-IR spectra**

FT-IR spectra of all the five organ samples without any drug treatments are shown in figure 3a for OH-stretching band and figure 3b for OH-bending band. The figures show distinct variation in normalized intensity and peak frequency for both OH-stretching and OH-bending bands.Figures 3a and b show highest intensity with lungs followed by brain, kidney, liver and stomach. Treatment effects of all the organ samples with related homeopathic potencies were shown in figure 4a for brain. The normalized spectra showed marked difference in intensity and peak frequency in both OH-stretching and OH-bending bands. In both cases the control (EtOH + *Hypericum* 200) showed higher intensity than the organ sample (Brain + *Hypericum* 200) (fig. 4a). Figure 4b shows the difference in intensity between the control (EtOH + *Merc cor* 30) and the organ sample (Kidney + *Merc cor* 30) for both OH-stretching and OH-bending bands. The OH-stretching and OH-bending bands showed differences in peak frequency (fig. 4b). Figure 4c shows the marked difference in intensity between the control (EtOH + *Phosphorus* 30) and organ sample (Lungs + *Phosphorus* 30). Here the organ sample (Lungs + *Phosphorus* 30) shows higher intensity than the control for both OH-stretching and OH-bending bands. The peak frequency showed difference for both OH-stretching and OH-bending bands (fig. 4c). Figure 4d shows the marked difference in intensity and peak frequency for both OH-stretching and OH-bending bands in case of control (EtOH + *Chelidonium* 200) and organ sample (Liver + *Chelidonium* 200). Here also the organ sample showed higher intensity than the control for both OH-stretching and OH-bending bands (fig. 4d). Figure 4e shows the marked difference in intensity and peak frequency for both OH-stretching and OH-bending bands in case of control (EtOH + *Cina* 30) and organ sample (Stomach + *Cina* 30). Here also the organ sample showed the higher intensity than the control for both OH-stretching and OH-bending bands (fig. 4e). The bands are very broad.

## DISCUSSION

Cut surfaces of organ samples, when put into the aqueous ethanol, established contact between the water structure in the organ samples and aqueous ethanol. In this way the solvent medium (Aqueous EtOH) takes up the water structure of the organ samples [3]. UV spectra of the water structure of five different organs show variation in intensity and number of peaks because the proteins and other biological contents are different in different tissues of

the organs. Homeopathic potencies can covert the water structure of a sample into a different form[8]. Since the water structures are different in different organs the effect of a homeopathic potency on the water structures of the organs should be different. This is evident in the UV spectra and the FT-IR spectra of the treated organ samples with related homeopathic potencies (fig. UV and FT-IR). In this way the right homeopathic remedy could produce appropriate effect on the water structure of an organ. It has been observed in both UV and FT-IR spectra that all potencies show distinct difference in intensity from their interaction with the water structure of organ samples. This shows that homeopathic potencies can change the water structure of the organ samples tested.

It was demonstrated that water structure is different in healthy and diseased tissues [9]. It is known that water structure plays an important role in protein function. All biomolecules are suspended in water and have specific structures for their biological functions. Tetrahedral hydrogen bonded molecules of water are present in the bulk solvent away from the hydration layer of protein present. In the hydration layer water molecules and polar atoms on the surface of protein form a three-dimensional chain connection of hydrogen bonded network. The networks are flexible and thus help in conformational changes of protein [4]. Cell membranes mediate many biological functions at the lipid water interface [10].

Water has a 4th state besides the three usual states of solid, liquid and vapour. The 4$^{th}$ state is structured water having crystalline clathrate hydrate structures [11]. It is the structured water which plays the most important role in homeopathic potencies. The only difference is that a homeopathic potency involves activated specifically structured water [1-2]. Ordinary structured water contains structure inducing agent, but a homeopathic potency removes these agents by serial dilution. A substance, may be a plant product, mineral, salt etc can induce specific structure in the water [12-13]. Besides a substance light, X-rays, magnate fields can also induce specific structures in water. Preparation of a homeopathic potency involves progressive serial dilution followed by mechanical agitation. Serial dilution results in loss of original substance molecules and produces a water structure which bears relation to the starting substance. There are evidences showing that many substances, X-rays, magnetic fields can change water structure [14-16]. Mechanical agitation changes hydrogen bonds, hydrogen bond strength, free water molecules. Aqueous ethanol solvent preserves the water structure in a homeopathic potency. Based on the results we can assume that a homeopathic potency can change water structure in the body of a patient including the affected part / organ. The potency, if properly selected, would positively interact with the water structure of the affected part / organ and thus initiate biochemical processes to restore health. Structured water can influence the physiology of man [17].

## CONCLUSIONS

Water structures in different organs like brain, kidney, liver, lungs, and stomach are different. A homeopathic potency related to an organ modifies the water structure of the organ. A homeopathic potency is activated specifically structured water which can modify selectively the water structure of the organ. It is possible that a properly selected homeopathic remedy may favourably modify the water structure of the affected organ, and initiate therapeutic effect in the concerned organ vis-à-vis the patient.

**CONFLICT OF INTEREST**

There is no conflict of interest.

**ETHICAL CONSIDERATIONS**

No animal was killed in the laboratory. The tested organ samples were collected from a local meat shop. For this, no ethical approval was needed.

## ACKNOWLEDGEMENT

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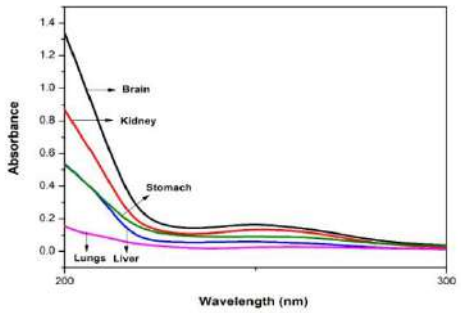



**Figure 1: UV spectra of water structures in 0.09% EtOH of 5 different organs of goat.**

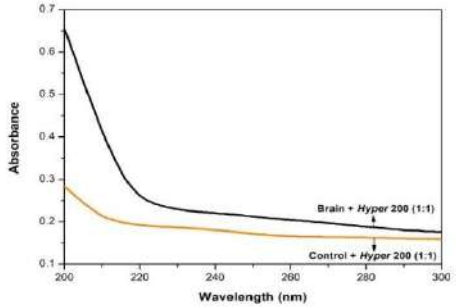



**Figure 2a: UV spectra of water structured in 0.09% EtOH of brain after treatment with *Hypericum* 200 in 0.09% EtOH. The drug mixed with water structure in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Hypericum* 200 in the proportion 1:1.**

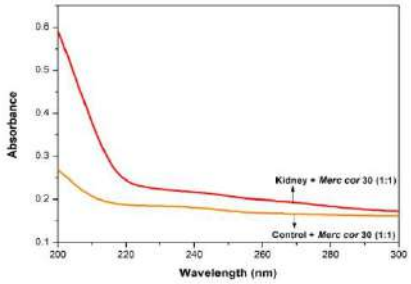



**Figure 2b: UV spectra of water structure in 0.09% EtOH of kidney after treatment with *Merc cor* 30 in 0.09% EtOH. The drug mixed with water structure in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Merc cor*30in the proportion 1:1.**

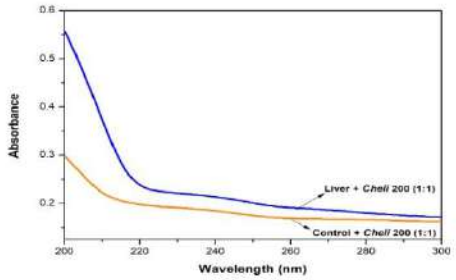



**Figure 2c: UV spectra of water structure in 0.09% EtOH of liver after treatment with *Chelidonium* 200 in 0.09% EtOH. The drug mixed with water structure in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Chelidonium*200 in the proportion 1:1.**

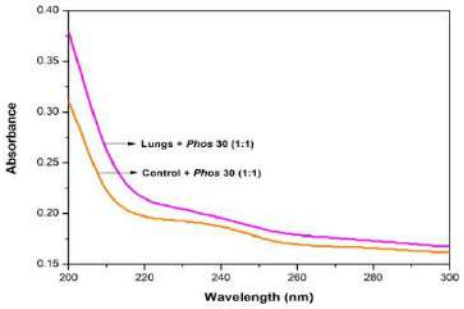



**Figure 2d: UV spectra of water structure in 0.09% EtOH of lungs after treatment with *Phosphorus* 30 in 0.09% EtOH. The drug mixed with water structure in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Phosphorus* 30 in the proportion 1:1.**

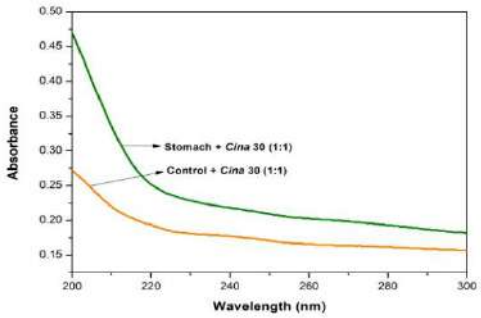



**Figure 2e: UV spectra of water structure in 0.09% EtOH of stomach after treatment with *Cina* 30 in 0.09% EtOH. The drug mixed with water structure in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Cina* 30 in the proportion 1:1.**

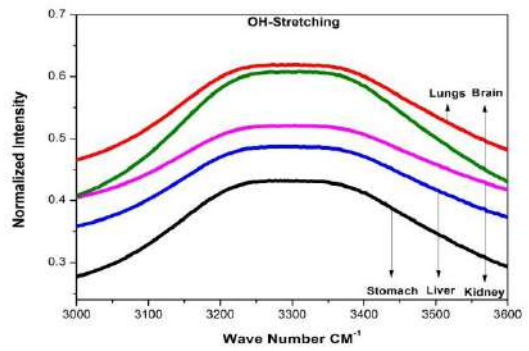



**Figure 3a: Normalized FT-IR spectra of water structures in 0.09% EtOH of 5 organs of goat showing OH-Stretching band.**

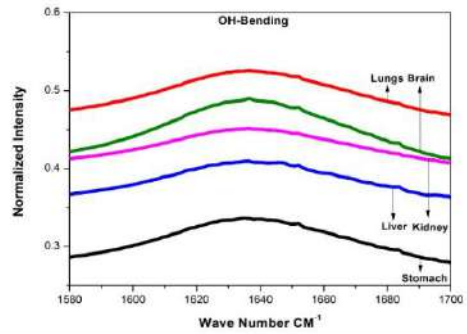
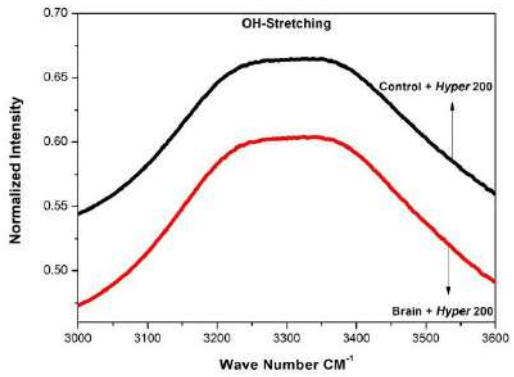



**Figure 3b: Normalized FT-IR spectra of water structures in 0.09% EtOH of 5 organs of goat showing OH-Bending band.**



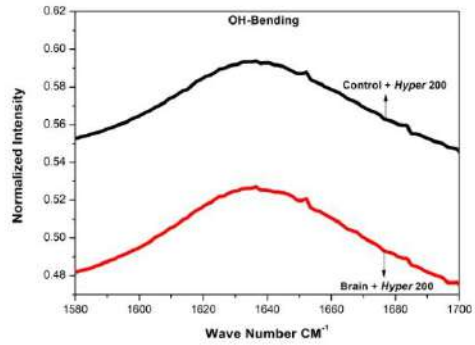
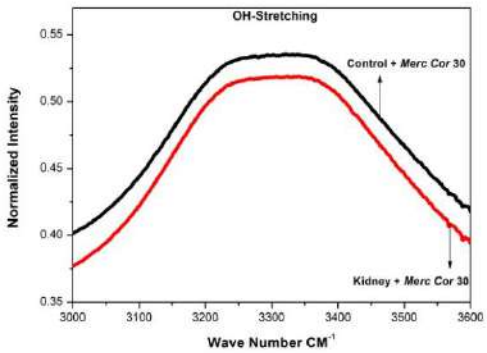




**Figure 4a: Normalized FT-IR spectra of water structure in 0.09% EtOH of brain of goat showing OH-Stretching (upper) and OH-Bending (lower) bands. The water structure was mixed with *Hypericum* 200 in 0.09% EtOH in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Hypericum* 200 in 0.09% EtOH in the proportion 1:1.**



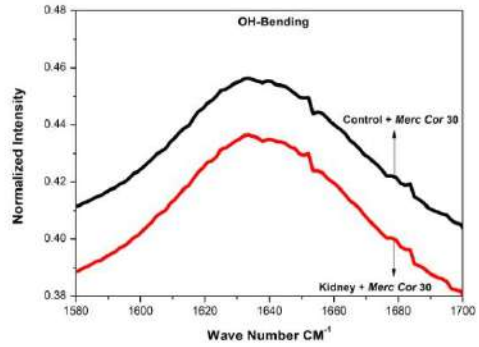




**Figure 4b: Normalized FT-IR spectra of water structure in 0.09% EtOH of kidney of goat showing OH-Stretching (upper) and OH-Bending (lower) bands. The water structure was mixed with *Merc cor* 30 in 0.09% EtOH in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Merc cor* 30 in 0.09% EtOH in the proportion 1:1.**

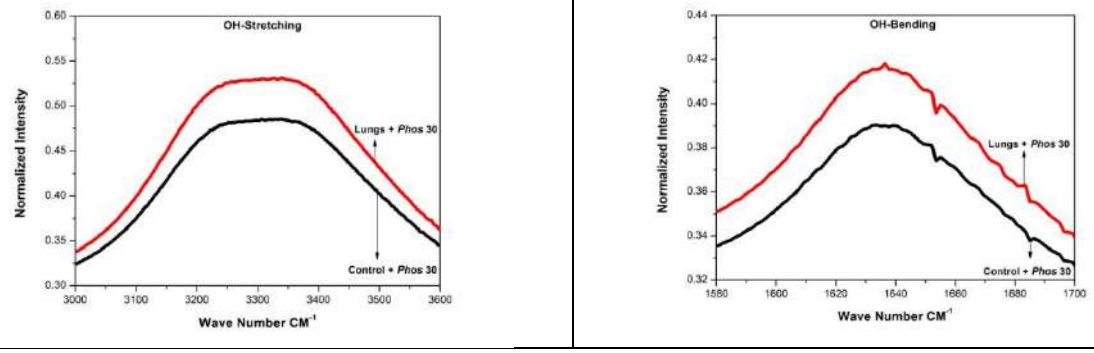



**Figure 4c: Normalized FT-IR spectra of water structure in 0.09% EtOH of lungs of goat showing OH-Stretching (upper) and OH-Bending (lower) bands. The water structure was mixed with *Phosphorus* 30 in 0.09% EtOH in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Phosphorus* 30 in 0.09% EtOH in the proportion 1:1.**

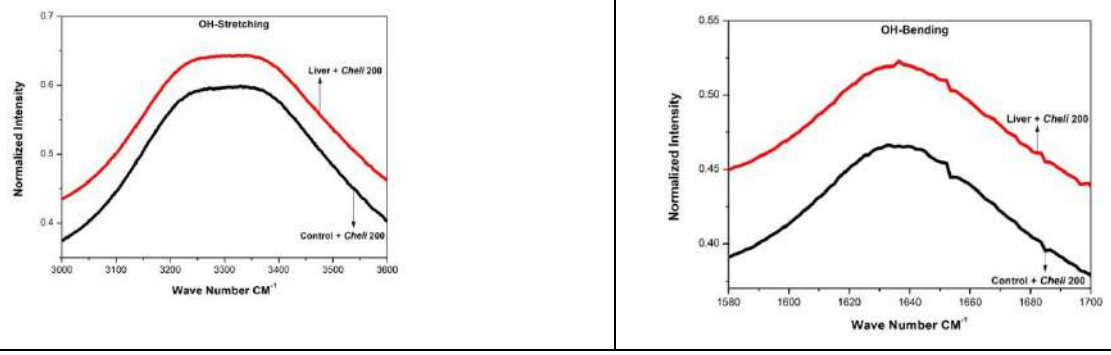



**Figure 4d: Normalized FT-IR spectra of water structure in 0.09% EtOH of liver of goat showing OH-Stretching (upper) and OH-Bending (lower) bands. The water structure was mixed with *Chelidonium* 200 in 0.09% EtOH in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Chelidonium* 200 in 0.09% EtOH in the proportion 1:1.**

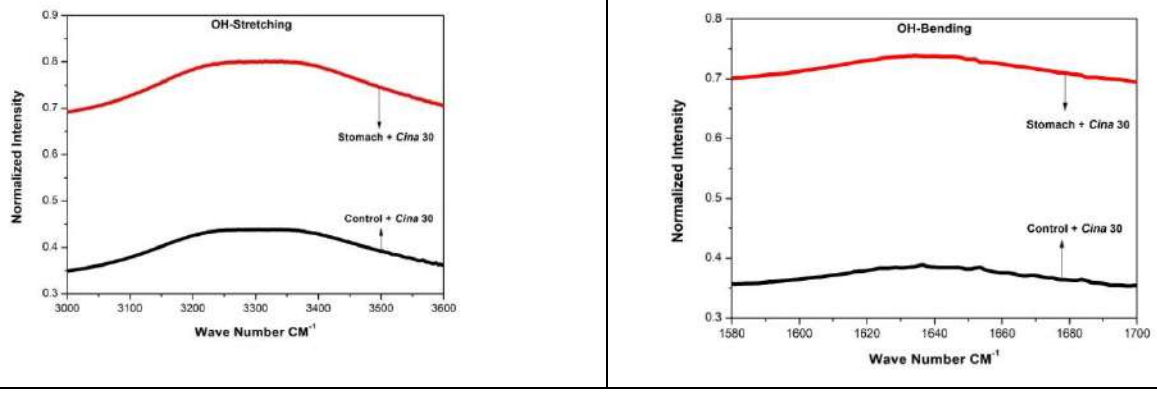



**Figure 4e: Normalized FT-IR spectra of water structure in 0.09% EtOH of stomach of goat showing OH-Stretching (upper) and OH-Bending (lower) bands. The water structure was mixed with *Cina* 30 in 0.09% EtOH in the proportion 1:1. The control consists of 0.09% EtOH mixed with *Cina* 30 in 0.09% EtOH in the proportion 1:1.**

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**RESEARCH ARTICLE**

# Detailed Pharmacognostic Standardization of *Gmelina asiatica* Linn. Fruit

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## ABSTRACT

*Gmelina asiatica* L., commonly known as Nilakumil in Tamil, is distributed throughout the state of Tamil Nadu, predominantly found in the coastal regions. In traditional medicine, the fruits of *Gmelina asiatica* L. have been used to treat conditions such as leucorrhoea, burning sensations in the eyes, and skin issues like eczema and dandruff. The primary objective of the present study is to standardize the pharmacognostical parameters, including macroscopy, microscopy, histo chemistry, physio-chemical, phytochemical analyses, and High-Performance Thin-Layer Chromatography (HPTLC) fingerprint. Macroscopic studies revealed that the fruit is dark orange-yellow, ovoid-oblong in shape, succulent, with a sweetish-slightly bitter taste. Microscopic investigations showed the presence of various structural components, including the epidermis, a narrow band of hypodermal cells, a wide mesocarp zone, and a stony endocarp. Powder microscopy revealed fragments of the pericarp, mesocarp, and reticulate parenchyma of the testa, endosperm with starch, cells of the cotyledon, sclereids, stone cells, and oil cells. Histochemical studies identified the presence of alkaloids, lignin, polysaccharides, tannin, cutin, and resin. Preliminary phytochemical analysis revealed the presence of alkaloids, phenols, tannins, flavonoids, triterpenoids, glycosides, steroids, and quinones. The moisture content was 9.38%, with alcohol and water-soluble extractive values of 48.45% and 52.03% respectively. The total ash content was 3.16%, while the acid-insoluble and water-soluble ash values were 0.19% and 52.03% respectively. Different extracts were subjected to HPTLC fingerprinting, which produced distinct peaks with varying

retention factor (Rf) values. These peaks serve as drug-specific identifiers. This study provides valuable guidance for accurately identifying the plant material and establishing standards for the crude drug.

**Keywords:** Adulteration, Crude Drug, *Gmelina asiatica* L. Fruit, Lamiaceae, Pharmacognosy.

## INTRODUCTION

Herbal remedies and plants have been utilized as medicine since ancient times. Over the last few decades, traditional medical systems have gained international attention. According to current estimates, a sizable section of the populace in many developing nations primarily receives their primary medical care from traditional healers and medicinal plants. At the same time, a growing number of people in wealthy nations are starting to use supplementary or alternative medicines, such as medicinal plants [1]. The demand for herbal drugs is now high and growing every day. Due to the abundance of readily available raw materials, the World Health Organization (WHO) supports and encourages the use of traditional herbs and cures in the healthcare industry. In nature, plants are incredibly complex. Their medicinal properties are influenced by species, geographic location, and methods of harvesting. Standardization of herbal drugs is crucial due to improper herb authentication, microbial adulterations, and pesticide residues. The WHO states that before any tests are conducted, the macroscopic and microscopic description of a medicinal plant is the first step towards confirming the identity and degree of purity of such materials [2].

The genus *Gmelina* was initially described by Linnaeus in 1753, based on the species *Gmelina asiatica* L. A total of 31 species and 2 subspecies are present in this genus [3]. *Gmelina asiatica* L. is known by various names in different Indian languages, such as Asian bush in English; *Bhadra* in Bengali; *Badhara*and *Nag-phul* in Hindi; *Guludu* and *Kalshivani*in Kannada; *Cherkumizhi* and *Kumilamaram* in Malayalam; *Lahan-shivan* in Marathi; *Nondano*and *Gopogombhari* in Orissa; *Badhara* in Punjab; *Gopabhadra, Biddari* and *Vikarini* in Sanskirit; *Nilakkumil, Nilakkimnizh, Nila-cumal; Kumizhaniaram, Kumil* and *Kadambal* in Tamil; *Chirugummudu, Gummadi, Cherunelli, Challagumudu* and *Shirigumudu* in Telugu [4]. It belongs to the Lamiaceae family and is found in dry evergreen to dry deciduous forests [5], growing as a scrambling shrub up to 8 meters high. *Gmelina asiatica* L. is widely utilized in Indian traditional medicine to treat jaundice, hemorrhoids, painful urination, arthritis, edema, liver diseases, neurological disorders, heart diseases, skin infections, acne, diabetes mellitus [6], Syphilis [7], dandruff [8], fever and rheumatism [9]. It also serves as an antiseptic, contraceptive [10], astringent, demulcent, bladder catarrh [7] and blood purifier [11]. Scientifically, the plant is reported to possess antibacterial [12-13], antifungal [12,13-14], antidandruff[15], anticancer [16], antioxidant[17-18],anti-inflammatory [14,19-20], antidiabetic, antihyperglycemic [21], antianxiety [22], antipyretic [23], Larvicidal [24], hepatoprotective[17] and nephroprotective [18].

1, 2-benzenedicarboxylic acid and monolinoleyl glycerol is the major bioactive compounds identified in the aerial parts of this plant [25]. The heartwood contains methyl p-methoxycinnamate, paulownin, gmelinol, cycloolivil, and sitosterol [26]. A flavone, ovalifolin, (+)-sesamin, sakuranetin, (-)-piperitol, and (+)-pinoresinol have been isolated from the alcoholic extract of the roots [27]. Palmitic, stearic, linoleic, oleic, and ricinoleic acids are reported in the seed oil[28].Kaempferol-3-rutinoside, apigenin-7-rutinoside, apigenin-7-glucuronide, and quercetagetin are identified in the flowers and leaves of *Gmelina asiatica* L. [29]. The fruits are edible [30] and used as a hair wash in traditional medicine [5]. Fresh fruits are boiled with coconut oil, made into a paste, and applied to the head to eliminate dandruff [31]. To date, no report is available on the pharmacognostic evaluation of *Gmelina asiatica* L. fruits. Therefore, this paper presents a report on the transverse section, powder microscopy, Histiochemistry, physicochemical parameters, preliminary phytochemical analysis, and High-Performance Thin-Layer Chromatography (HPTLC) of the fruit of *Gmelina asiatica* L.

## MATERIALS AND METHODS

**Collection and Identification of Plant Material**

The fresh fruits of *Gmelina asiatica* L. were collected from Cuddalore district, Tamil Nadu. The plant's taxonomic identity was confirmed by referencing the floristic work conducted by earlier researchers [32-33] and consulting the Madras Herbarium (MH). A voucher specimen B.S.I. (S.R.C) No. 150401 has been deposited in the Madras Herbarium, Botanical Survey of India, Southern Regional Centre, Coimbatore.

**Botanical Description**

*Gmelina asiatica* L., Sp. pl. 626.1753; Roxb. Fl. ind.3: 87. 1832; Wight, III.Ind.Bot. t. 174. 1850; Hook.f. FI. Brit. India 4: 582. 1885; Gamble, Fl. Madras 2:1098 (768). 1924; Matthew, FTNC 1: 301.1981, 3: 1222. 1983, 4: t.470.1988& FPH 968. 1999; COL & WCSP 2018; III.Fl. C&NTN t. 1685.2019. *G.Parvifolia* Roxb. Pl. Coromandel t. 162. 1802 (Figure 1). Large straggling deciduous shrub, rarely tree, 3-4 m high; young twigs minutely hairy; spines to 2.5 cm; Leaves ovate to elliptic, 2.5 × 1 cm, apex obtuse to acute; base rounded to cuneate, margin entire to irregularly lobed; upper surface glabrous, dark green, shiny; lower surface glabrous, whitish with peltate scales; petiole to 1 cm. Inflorescence – Racemes axillary and terminal to 9 cm. Calyx 4 lobed, puberulous without; 0.4 × 0.3 cm; lobes acute, 0.1 cm long. Corolla golden yellow; 4 lobed, anterior lip 3- lobed; posterior lip 1- lobed. Stamens 4, inserted at apex of the tube, glabrous; anterior pair 2.3 cm long, anthers 0.2 cm long; posterior pair fertile, 1.1 cm long, anthers 0.1 cm long. Ovary 0.4 cm, glabrous; style 2.6 cm long. Fruit 3 × 1 cm, glabrous, apex rounded, succulent drupe yellow when ripe. Seeds 2, oblong, 1.2 × 0.5 cm. Flowering and Fruiting: Throughout the year.

**Macro-Microscopy**

The fruits were soaked in water for 12 hours, and free-hand transverse sections were taken according to standard procedures [35-37]. A Zeiss Stereo Discovery V.8 microscope connected with an Axiocam ERc5s was used to examine macro-morphological features, and various characteristics of the fruit, such as color, shape, size, odor, and taste, were recorded. A Zeiss Axiolab 5 microscope with an Axiocam 208 color camera was used to observe micro-morphological features.

**Powder Microscopy**

The samples were cleaned, shade dried, powdered, and passed through sieve No. 80. The powder was separately treated with glycerine (50%, v/v) and chloral hydrates (10%, v/v). Then the Sample was stained with iodine solution to confirm the presence of starch grains and the characters were observed and photographed under different magnifications by using a Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light.

**Histochemistry**

Transverse sections were stained using specific histo-chemical procedures [37].

**Preparation of Fruit Extract**

The fresh fruits were washed with water, dried in shade, and powdered. Four grams of the powder were extracted with 100 ml of n-hexane in a Soxhlet apparatus. The extract was concentrated using a rotary evaporator under reduced pressure (100 mbar) and reduced temperature (55°C). It was then dissolved in 10 ml of n-hexane. The marc in the thimble was successively extracted with hexane, chloroform, and ethanol. Their extracts were redissolved in the respective solvents and used for preliminary phytochemical analysis and HPTLC analysis.

**Physio-chemical Parameters**

Physiochemical parameters such as Loss on drying, ash values, and extractive values, were carried out as per the standard procedure [38].

**Phytochemical Screening**
The phytochemical analysis was performed using standard procedure [39].

**HPTLC fingerprinting analysis:**
Thirty microliters (30 µl) of the extracts were applied to a silica-coated TLC plate $60F_{254}$ using Camag's ATS4 sample applicator and developed in a twin trough chamber (CAMAG) measuring 20 × 10 cm, previously saturated with the mobile phase. The mobile phase for the hexane extract was Toluene: Ethyl acetate: Formic acid (9.5:0.5:0.5, v/v/v); for chloroform and ethanol extracts, it was Toluene: Ethyl acetate: Formic acid (7:3:0.5, v/v/v). The developed plate was dried over a hot plate and a photograph was taken in CAMAG Visualizer Chamber followed by scanning under λ254 (absorbance mode, D2 lamp) and λ354 (Fluorescence mode, Hg lamp) respectively with a slit dimension 6×0.45 mm and scanning speed of 20 mm/s by scanner 4 (Scanner_210441) linked with WINCATS software. The scanned plate was dipped in Vanillin sulphuric acid (VSR) and heated at 105°C till the appearance of colored bands. The photograph was taken immediately at white light followed by scanning at λ 520 at absorption mode (W lamp).

## RESULTS AND DISCUSSION

In the herbal medicine sector, incorrect plant species identification has resulted in adulteration and raw drug replacement [40]. Accurate identification, quality assurance, and the development of pharmacognostic standards are necessary for an evaluation of medicinal plants. The WHO states that evaluating a medical plant both macroscopically and microscopically is the first step towards plant standardization [41-42]. Microscopic characters have a lot of potential applications in authentication on several levels. Even though scientists believe that it can be challenging to distinguish between closely related genera within a family based solely on anatomical traits, careful cellular analysis or powder microscopy may be able to help resolve the authentication problems [43-44].

**Macroscopic Evaluation**
The fruit is ovoid, oblong, or pyriform in shape and succulent, the surface is smooth glossy, and dark orange-yellow in colour. 1- 2 seeded drupe. Calyx is persistent, attached at the base of the pointed end of the fruit a short pedicel lies at the centre of it, and a small depression is occasionally located at the broader end, 1.5 to 2 cm in length, 0.5 to 1 cm in width and 0.5 to 0.8 mm in thickness. Pericarp is sweetish-slightly bitter and mucilaginous; odour characteristic, taste oily (Figure 2).

**Microscopic Evaluation**
**Fruit**
The transverse section of the fruit pulp (Figure 3) shows three layers i.e., epicarp, mesocarp, and endocarp. The epicarp region consists of a single layer of small rectangular cells arranged radially. The epidermis is covered with a thin layer of cuticle. Followed by the epidermis, 10 to 15 rows of isodiametric, spherical hypodermal cells comprise parenchymatous. The mesocarp is fleshy, consisting of a wide zone of parenchymatous cells arranged in numerous layers approximately 15 to 20 layers. Outer mesocarp cells are isodiametric, whereas inner mesocarp are radially elongated and closely packed, narrow cells. Vascular strands are embedded in the inner side of it, tangentially running celled layer of inner epidermis lies underneath this, followed by a broad zone of sclerenchymatous band of endocarp.

**Seed**
The transverse section of the seed shows narrow, small-sized cells of the outer epidermis followed by inconspicuous endosperm consisting of 1 to 2 rows of tangentially running cells embedded with aleurone grains, cotyledons consisting of outer and inner epidermis enclosing the wide mesophyll tissue embedded with fixed oil and aleurone grains (Figure 4).

**Powder Microscopy**

The fruit powder is dark brown in color with a slightly aromatic odor and characteristic taste. It shows fragments of the pericarp, mesocarp, and reticulate parenchyma of the testa, endosperm with starch, cells of the cotyledon, sclereids, stone cells, cells with content, and oil cells (Figure 5).

**Histochemistry**

In the fruit pericarp, alkaloids were present in the epicarp and mesocarp cells; cutin and resin were noted in the epidermal cells; lignin and mucilage were present in the mesocarp region, while tannin deposition was present in both the epicarp and mesocarp regions as shown in (Figure 6). In the seed, alkaloids and aleurone were present in the cotyledonary cells; lignin deposition was observed in the testa cells; oil droplets were observed in the endosperm cells, and tannin was present in the cotyledon cells (Figure 7).

With the use of particular staining reactions and imaging techniques, histochemistry enables the identification and localization of biomolecules and organelles in many types of cells and tissues at the cellular level. The in vivo localization of promoters in certain tissues and the identification of particular cell wall constituents like polysaccharides and lignin are two other common uses for histochemical techniques [45].The chemical histo-localisation allows determining the exact storage site of the secondary metabolites.

**Physio-Chemical Parameters**

The physio-chemical parameters of powdered fruit pulp of *Gmelina asiatica* L. were evaluated in Table 3. Physicochemical parameters play a crucial role in standardizing and ensuring the quality of herbal drugs. These parameters encompass various factors such as loss on drying, ash content, and pH. Herbal materials need to be free from contamination, making foreign matter analysis of powdered drugs a significant parameter for assessing the purity of herbal drugs [46]. The loss on drying test is a widely employed method to determine the moisture content in powdered samples. It's essential for the moisture content of drugs to be kept at minimal levels to prevent the growth of microbes during storage [47]. Ash values are utilized to assess the quality and purity of crude drugs, indicating the presence of various impurities such as carbonate, oxalate, and silicate. Water-soluble ash is employed to gauge the quantity of inorganic compounds in drugs, while acid-insoluble ash primarily consists of silica, indicating contamination with earthy materials [47]. Estimating extractive values helps determine the quantity of active constituents present in a given amount of plant material when it's extracted with a specific solvent. When any crude drug is extracted with a particular solvent, it yields a solution containing various phytoconstituents. The composition of these chemical constituents is influenced by both the nature of the drug and the solvent utilized. Additionally, this process offers an indication of whether the crude drug has been fully depleted or not[46-48].

**Preliminary Phytochemical Screening**

The preliminary phytochemical screening of *Gmelina asiatica* L. fruit pulp revealed the presence of diverse phytochemical compounds, which can be found in Table 2.Alkaloids, Phenols, tannins, flavonoids, triterpenoids, glycosides, steroids, quinones, and cardiac glycosides constituted the phytoconstituents in hexane, chloroform, and ethanol extracts. However, proteins, reducing sugars, coumarin, and anthraquinones, acids were not found in ethanol, chloroform, and hexane extracts. Among the three extracts, the hexane extract exhibited significantly lower levels of phytoconstituents

**HPTLC Fingerprint Analysis**

The results of the Thin Layer Chromatography (TLC) photodocumentation of 30 microliters of different extracts under UV 254 nm, 366 nm, and white light after derivatization using vanillin-sulphuric acid reagent at 520 nm are shown in Figure 8 and Table 3. The HPTLC fingerprint profiles of different extracts of the fruits pulp of *Gmelin asiatica* L. are presented in Figures 9 to 11. The HPTLC analysis of the hexane extract shown in Figure 9 reveals that under UV 254 nm, eight peaks are observed, with the major peaks present at Retention factor (Rf) values of 0.90 (45.89%), 0.94 (16.14%), and 0.82 (15.42%). Under UV 354 nm, the major peaks are found at Rf values of 0.44 (41.48%), 0.28 (24.63%), and 0.96 (24.46%). After derivatization with vanillin-sulphuric acid, at white light 520 nm, the major

peaks are observed at Rf values of 0.97 (25.94%), 0.29 (22.14%), and 0.44 (18.33%). In the chloroform extract, twelve peaks were recorded, with the major peaks seen at Rf values of 0.94 (52.20% area) and 0.58 (14.91% area) under UV 254 nm. In the ethanol extract, under UV 254 nm, five peaks were identified, with the major peaks observed at Rf values of 0.33 (30.8% area), 0.95 (25.88% area), and 0.55 (18.79% area). Under $\lambda$ 354 nm, a total of two peaks were recorded, with the major peaks appearing at an Rf value of 0.91 (82.37% area). After derivatization with vanillin-sulphuric acid and examining at white light 520 nm, a total of eight peaks were recorded, with the major peaks found at Rf values of 0.51 (20.75% area), 0.75 (17.97% area), and 0.46 (13.77% area), as presented in Figure 11.This finding is useful for supplementing existing information on the identification and standardization of *Gmelina asiatica* L., even in its powdered form, to distinguish it from other drugs and adulterants. The HPTLC analysis conducted for the chemical profiling of *Gmelina asiatica* L. fruit will be useful in identifying bioactive chemicals and markers by a comparison of the compound's Rf values with reference standards. These findings also imply that the pharmacognostic and physiochemical parameters that are observed have a significant role in formulation development and quality control of the crude drug.

## CONCLUSION

Pharmacognostic standardizations is essential to determining a crude drug's accurate identity and quality. Prior to any medication being added to the pharmacopoeia, these requirements need to be met. The macroscopy, microscopy, physiochemical, and phytochemical aspects of the plant material provide the majority of the information regarding its identity, purity, and quality. Since there is no documentation of pharmacognostical research on *Gmelina asiatica* L. fruits, the current study was conducted to establish essential phytopharmacopoecial standards. Further, it could attract the attention of pharmacologists to explore this plant through scientific research.

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**Table 1. Physicochemical parameters of *Gmelina asiatica* L. fruit pulp**

| Name of the Parameters | Mean value |
|---|---|
| Moisture content at 105°C(%, w/w) | 9.38 |
| Total ash (%, w/w) | 3.16 |
| Acid insoluble ash (%, w/w) | 0.19 |
| Water soluble extractive (%, w/w) | 52.03 |
| Alcohol soluble extractive (%, w/w) | 48.45 |
| pH value (10% solution) | 4.63 |

**Table 2. Preliminary phyto chemical screening of *Gmelina asiatica* L. fruit pulp extracts.**

| S. No | Tests for phyto constituents | Hexane | Chloroform | Ethanol |
|---|---|---|---|---|
| 1. | Alkaloids | - | - | + |
| 2. | Flavonoids | - | + | + |

| | | | | |
|---|---|---|---|---|
| 3. | Tannins | - | + | + |
| 4. | Phenol | + | + | + |
| 5. | Saponins | - | - | + |
| 6. | Triterpenoids | + | + | + |
| 7. | Proteins | - | - | - |
| 8. | Glycosides | - | + | + |
| 9. | Reducing sugar | - | - | - |
| 10. | Anthraquinones | - | - | - |
| 11. | Quinones | - | + | + |
| 12. | Cardiac glycoside | - | - | + |
| 13. | Steroids | + | + | + |
| 14. | Coumarin | - | - | - |
| 15. | Acids | - | - | - |
| Abbreviations: **+**, present; **-**, absent. | | | | |

**Table 3.Presents the Retention factor (Rf) values obtained from TLC profiling of extracts derived from *Gmelina asiatica* L. Fruit pulp.**

| Name of the extract | UV 254 nm | | UV366 nm | | After derivatizing with 5% Vanillin sulphuric acid reagent | |
|---|---|---|---|---|---|---|
| | Rf | Colour | Rf | Colour | Rf | Colour |
| **n-Hexane** | 0.05 | Green | 0.05 | Lightred | 0.08 | Light violet |
| | 0.29 | Green | 0.10 | Lightred | 0.17 | Purple |
| | 0.35 | Green | 0.29 | Red | 0.30 | Purple |
| | 0.44 | Green | 0.39 | Blue | 0.40 | Light purple |
| | 0.61 | Green | 0.45 | Red | 0.44 | Purple |
| | 0.82 | Green | 0.69 | Lightred | 0.55 | Purple |
| | 0.91 | Green | 0.76 | Lightred | 0.61 | Purple |
| | 0.98 | Green | 0.91 | Blue | 0.65 | Light purple |
| | | | 0.98 | Blue | 0.71 | Light purple |
| | | | | | 0.90 | Violet |
| | | | | | 0.94 | Violet |
| **Chloroform** | 0.08 | Green | 0.12 | Blue | 0.10 | Grey |
| | 0.10 | Green | 0.21 | Blue | 0.17 | Purple |
| | 0.14 | Green | 0.32 | Blue | 0.26 | Yellow |
| | 0.21 | Green | 0.46 | Blue | 0.35 | Yellow |
| | 0.31 | Green | 0.55 | Blue | 0.42 | Brown |
| | 0.39 | Green | 0.69 | Blue | 0.47 | Yellow |
| | 0.47 | Green | 0.75 | Lightgreen | 0.50 | Yellow |
| | 0.52 | Green | 0.79 | Lightred | 0.58 | Light yellow |
| | 0.58 | Green | 0.86 | Light pink | 0.73 | Pink |
| **Ethanol** | 0.09 | Green | 0.10 | Blue | 0.06 | grey |
| | 0.38 | Green | 0.19 | Blue | 0.17 | Light pink |
| | 0.45 | Green | 0.35 | Blue | 0.21 | Light pink |
| | 0.58 | Green | 0.46 | Blue | 0.27 | Yellow |
| | | | 0.64 | Blue | 0.39 | Light purple |
| | | | 0.80 | Green | 0.44 | Yellow |
| | | | 0.89 | Blue | 0.47 | Pink |
| | | | | | 0.58 | Yellow |
| | | | | | 0.78 | Pink |

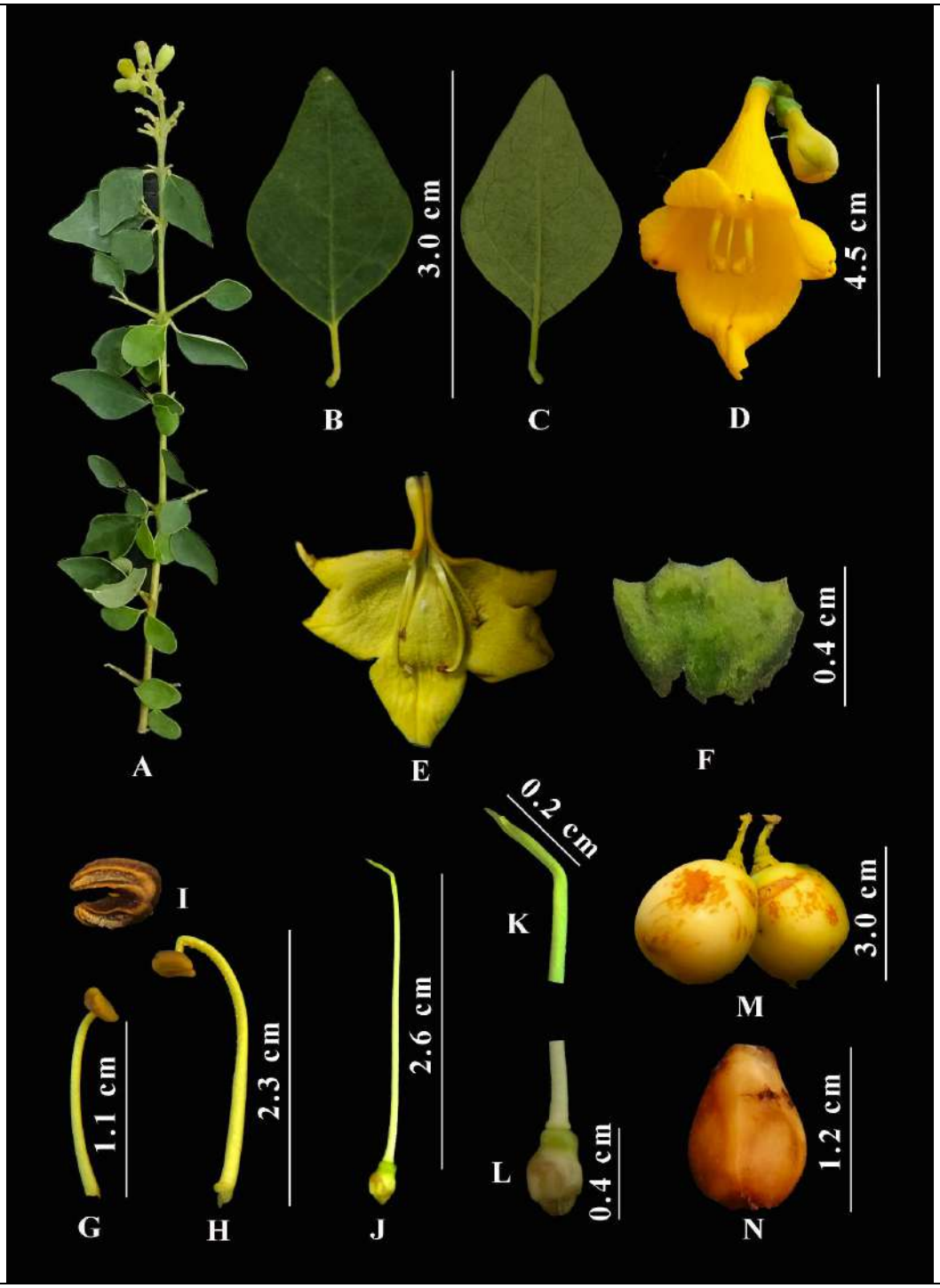



**Figure 1.***Gmelina asiatica* **L., A) A portion of twig; B) Adaxial surface of leaf; C) Abaxial surface of leaf; D) Inflorescence; E) Corolla -split out; F) Calyx; G) Smaller stamen; H) Larger stamen; I) Anther- enlarged view; J) Gynoecium; K) Stigma-enlarged view; L) Ovary; M) Fruits; N) Seed**

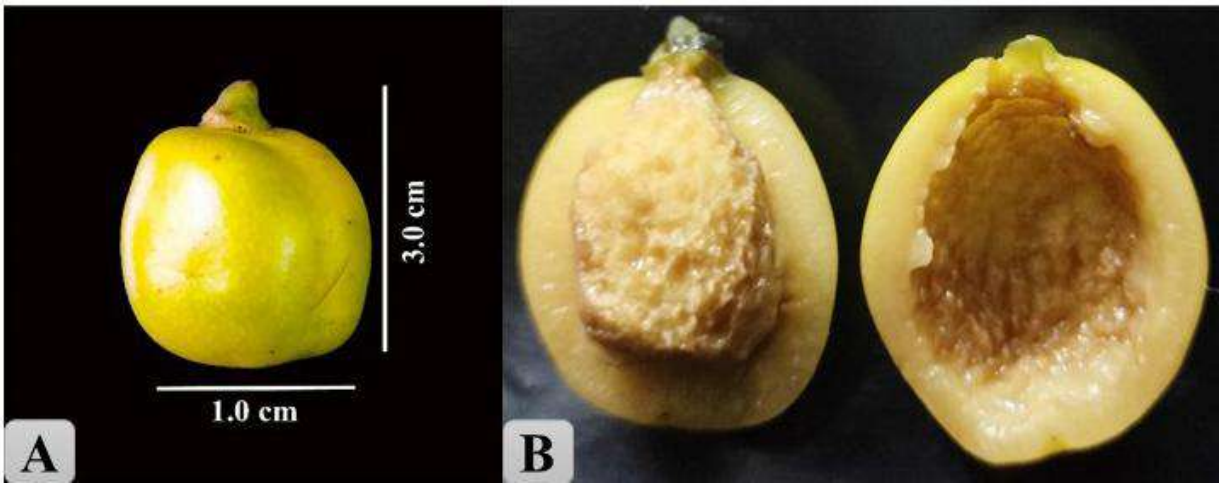



**Figure 2. Macroscopic character of *Gmelina asiatica* L. Fruit. A. Whole Fruit; B. Longitudinal split up of fruit.**

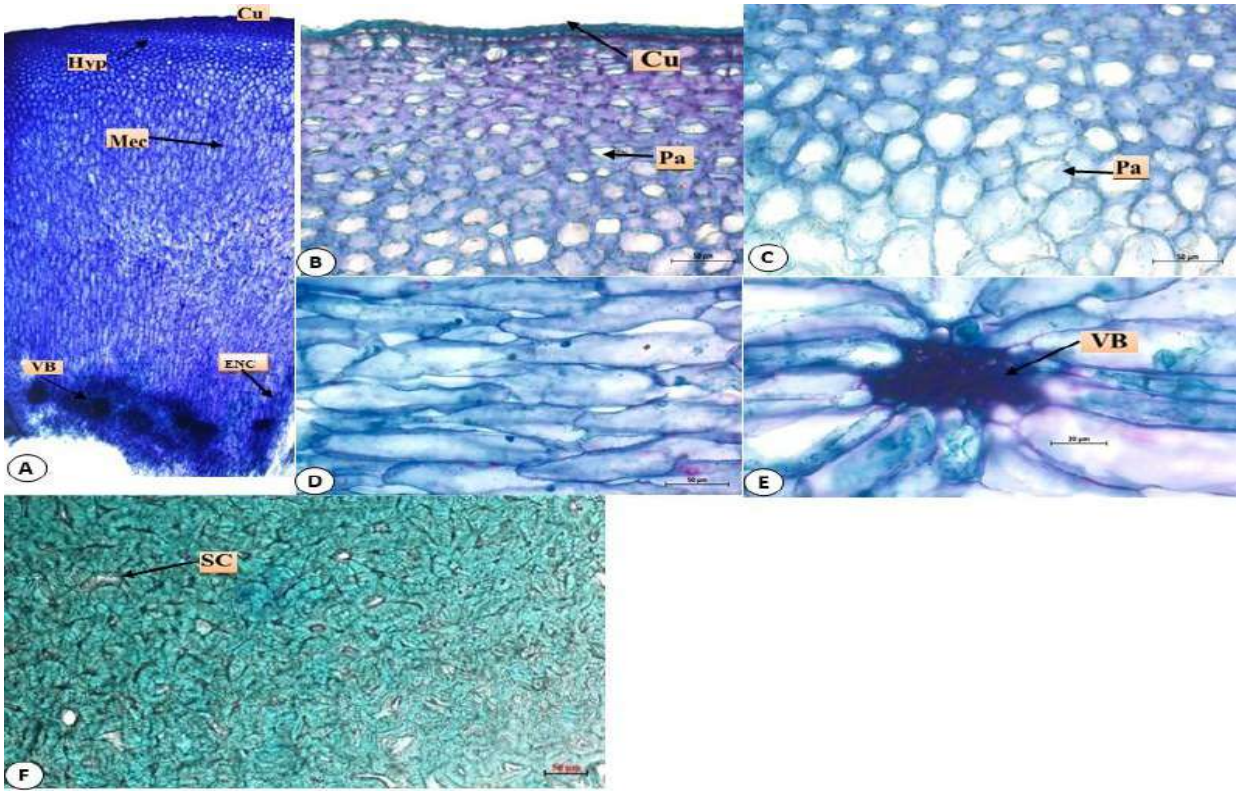



**Figure 3. Anatomy of *Gmelina asiatica* L. fruit pulp. A. Transverse section of fruit; B. outer region enlarged; C. inner mesocarp cells; D. outer mesocarp cells; E. vascular bundles in the mesocarp cells; F. stony endocarp.**

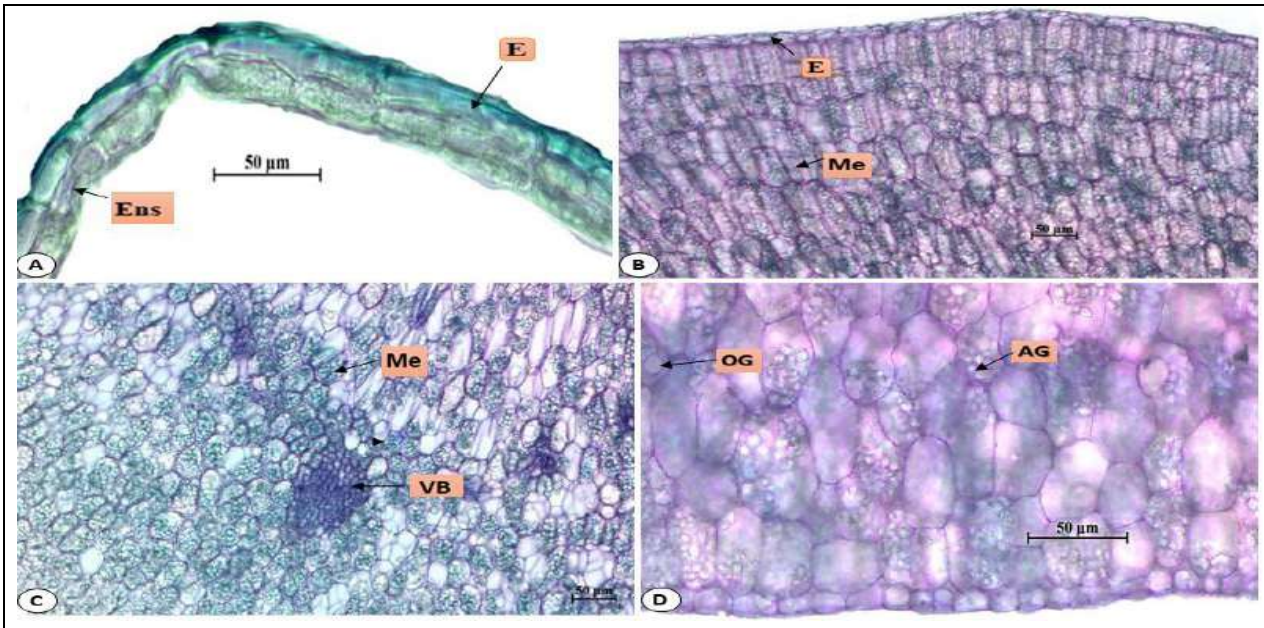



**Figure 4.Transverse section of *Gmelina asiatica* L. Seed. A. Testa and endosperm; B. upper region enlarged; C. middle region enlarged; D. lower region enlarged.**

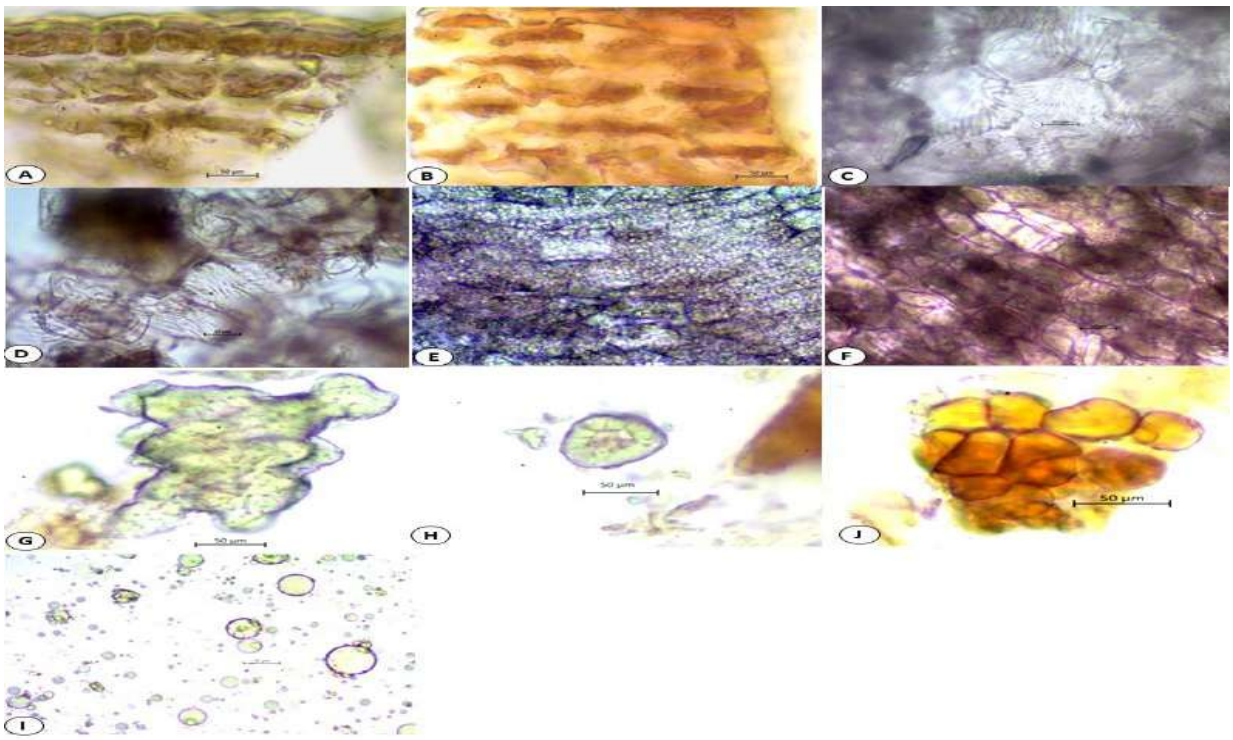



**Figure 5. Powder microscopy of *Gmelina asiatica* L. fruit. A. pericarp; B. mesocarp; C and D. cells of the endocarp; E. endosperm cells with starch; F. cotyledon cells; G. sclereids; H. stone cells; I. cells with brownish content; J. oil drops.**

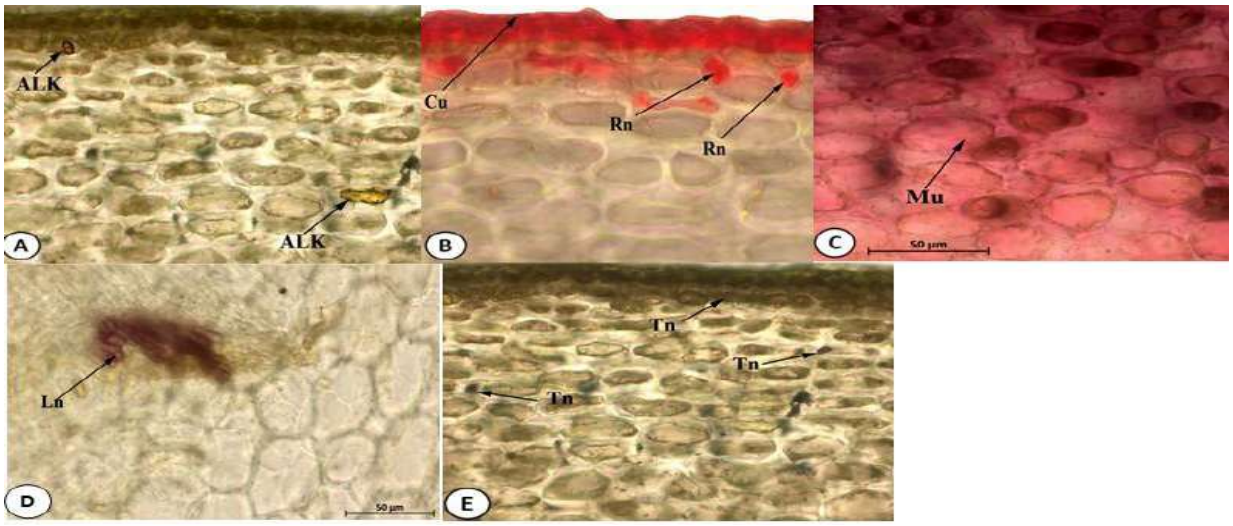



**Figure 6. Histo chemistry of fruit pericarp. A. alkaloid; B. cutin and resin; C. mucilage; D. lignin; E. tannin**

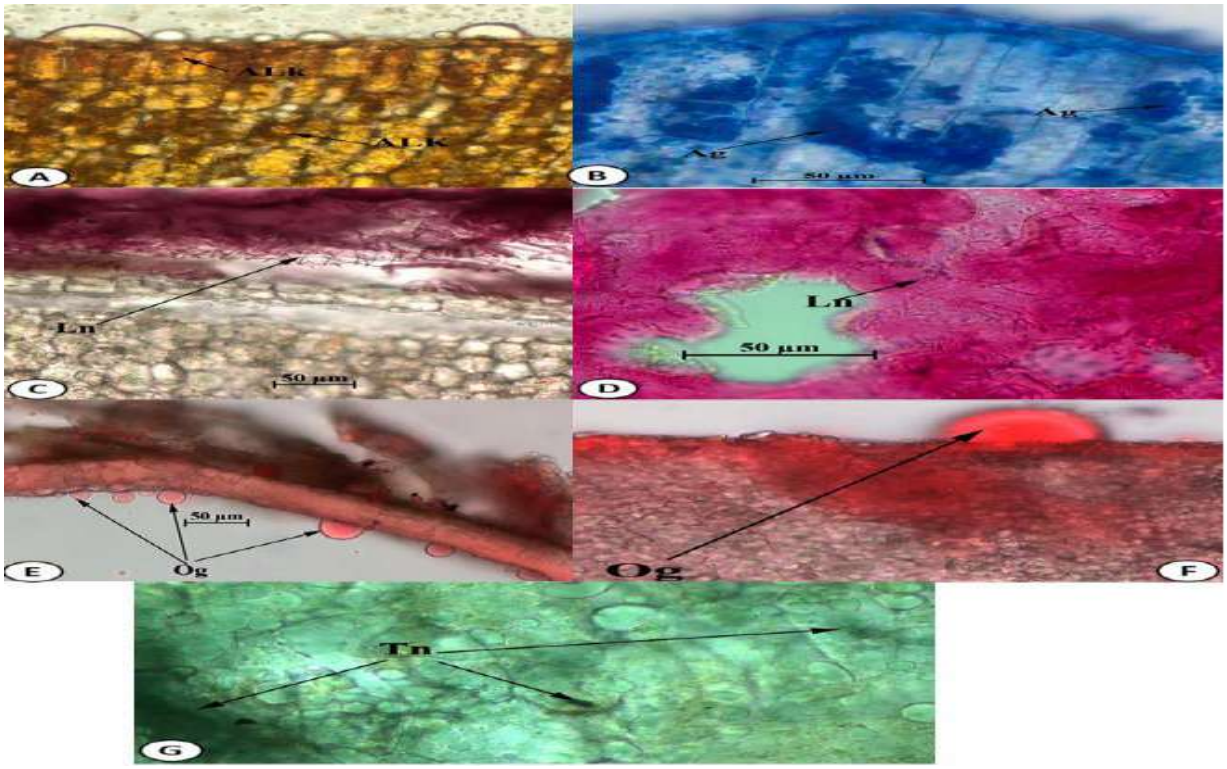



**Figure 7. Histochemistry of *Gmelina asiatica* seed. A. alkaloid; B. aleurone grains; C and D. lignin; E and F. oil globule; G. tannin.**

**Abbreviations: AG-aleurone grains; ALK- alkaloid; Cu- cutin; Cut- cuticle; E-epidermis; Enc-endocarp; Ens- endosperm; Hyp-hypodermis; Ln- lignin; Me- mesophyll; Mec- mesocarp; Mu- mucilage; OG-oil globules; Pa-parenchyma; Rn- resin; Sc-stony endocarp; Tn- tannin; VB-vascular bundle.**

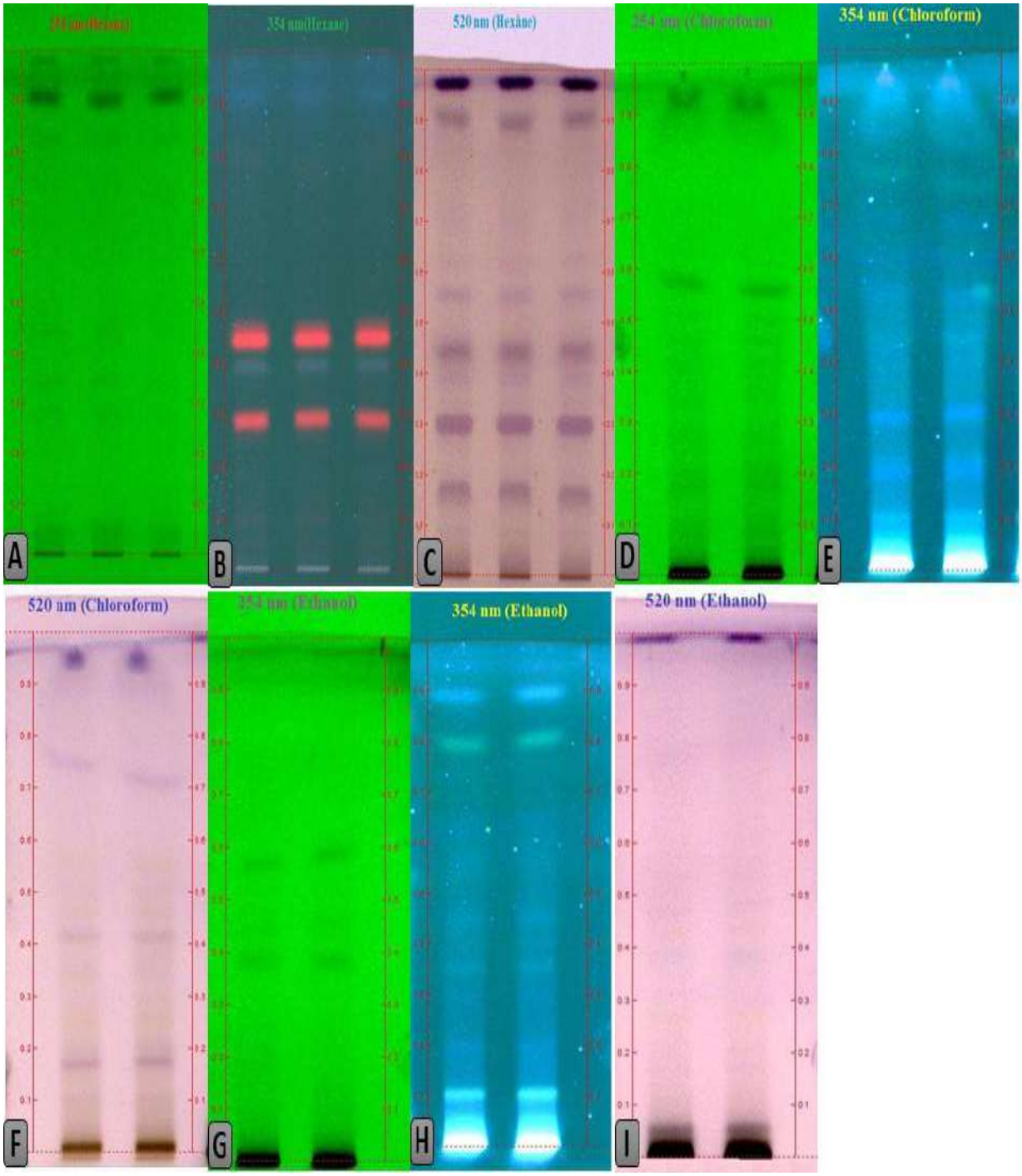
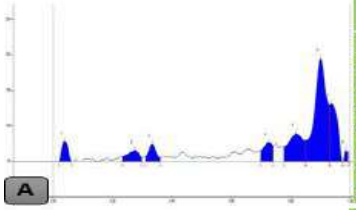
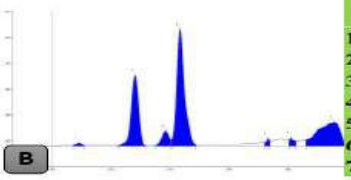


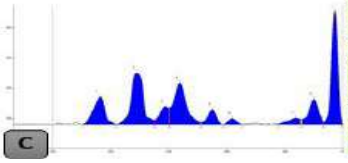

**Figure 8. TLC Photo documentation of *Gmelina asiatica* L fruit pulp. A-C: Hexane extract; D-F: Chloroform extract; G-I: Ethanol extract.**




| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.02 | 0.4 | 0.04 | 27.5 | 7.33 | 0.06 | 0.3 | 517.1 | 4.62 |
| 2 | 0.23 | 6.8 | 0.28 | 15.8 | 4.20 | 0.30 | 5.8 | 556.1 | 4.97 |
| 3 | 0.31 | 6.9 | 0.33 | 24.7 | 6.59 | 0.36 | 5.5 | 558.2 | 4.99 |
| 4 | 0.70 | 15.4 | 0.73 | 26.5 | 7.07 | 0.74 | 22.7 | 744.6 | 6.65 |
| 5 | 0.78 | 20.5 | 0.82 | 39.7 | 10.56 | 0.85 | 29.4 | 1726.3 | 15.42 |
| 6 | 0.85 | 29.5 | 0.90 | 144.8 | 38.57 | 0.93 | 78.8 | 5136.7 | 45.89 |
| 7 | 0.93 | 79.1 | 0.94 | 80.7 | 21.48 | 0.97 | 0.3 | 1806.8 | 16.14 |
| 8 | 0.98 | 1.2 | 0.99 | 15.8 | 4.21 | 0.99 | 13.9 | 147.7 | 1.32 |

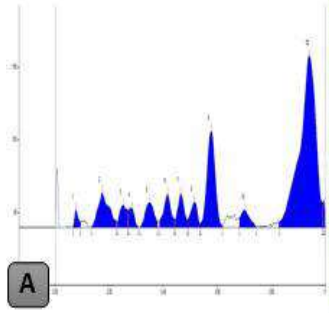



| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.07 | 4.3 | 0.09 | 12.9 | 1.35 | 0.11 | 3.6 | 268.2 | 1.06 |
| 2 | 0.23 | 2.9 | 0.28 | 273.9 | 28.65 | 0.32 | 3.8 | 6250.4 | 24.63 |
| 3 | 0.35 | 5.0 | 0.39 | 59.2 | 6.19 | 0.41 | 24.2 | 1342.4 | 5.29 |
| 4 | 0.41 | 25.0 | 0.44 | 452.1 | 47.30 | 0.49 | 7.9 | 10526.0 | 41.48 |
| 5 | 0.72 | 15.2 | 0.74 | 30.7 | 3.21 | 0.74 | 18.4 | 317.2 | 1.25 |
| 6 | 0.81 | 22.5 | 0.81 | 33.0 | 3.46 | 0.83 | 19.4 | 461.9 | 1.82 |
| 7 | 0.86 | 23.7 | 0.96 | 94.1 | 9.84 | 1.00 | 7.3 | 6207.5 | 24.46 |

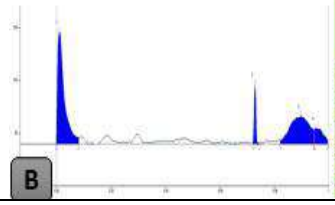



| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.11 | 0.5 | 0.17 | 91.4 | 9.12 | 0.22 | 0.4 | 3152.9 | 10.91 |
| 2 | 0.22 | 0.2 | 0.29 | 170.4 | 17.00 | 0.35 | 13.9 | 6399.1 | 22.14 |
| 3 | 0.35 | 14.0 | 0.39 | 57.7 | 5.75 | 0.40 | 53.2 | 1574.6 | 5.45 |
| 4 | 0.40 | 53. 4 | 0.44 | 137.1 | 13.67 | 0.51 | 7.2 | 5299.5 | 18.33 |
| 5 | 0.51 | 7.2 | 0.55 | 49.7 | 4.95 | 0.59 | 0.1 | 1290.9 | 4.47 |
| 6 | 0.59 | 0.0 | 0.62 | 19.0 | 1.89 | 0.65 | 0.0 | 452.2 | 1.56 |
| 7 | 0.78 | 0.7 | 0.84 | 20.4 | 2.04 | 0.86 | 16.9 | 724.5 | 2.51 |
| 8 | 0.86 | 17.0 | 0.90 | 83.2 | 8.30 | 0.93 | 7.2 | 2512.9 | 8.69 |
| 9 | 0.94 | 7.3 | 0.97 | 373.7 | 37.28 | 1.00 | 6.6 | 7499.6 | 25.94 |

**Figure 9. HPTLC fingerprint profile of n-Hexane extract of *Gmelina asiatica* L fruit pulp. a) UV 254 nm. b) UV 354 nm. c) long white light 520 nm.**

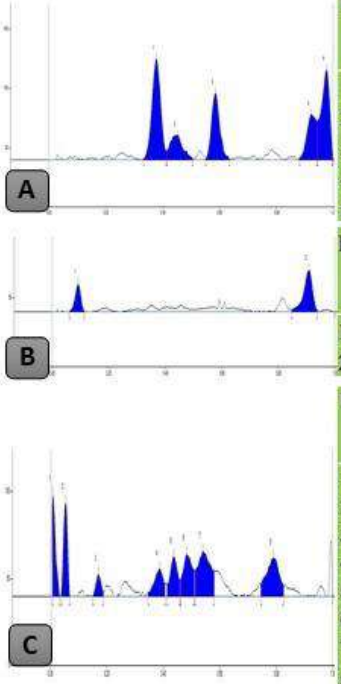



| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.07 | 0.1 | 0.08 | 12.1 | 3.38 | 0.09 | 3.9 | 142.7 | 1.21 |
| 2 | 0.13 | 0.4 | 0.17 | 23.7 | 6.63 | 0.23 | 4.3 | 838.4 | 7.13 |
| 3 | 0.23 | 4.0 | 0.25 | 15.0 | 4.20 | 0.27 | 11.7 | 359.1 | 3.05 |
| 4 | 0.27 | 11.7 | 0.29 | 13.0 | 3.65 | 0.31 | 0.3 | 236.1 | 2.01 |
| 5 | 0.31 | 0.0 | 0.35 | 16.8 | 4.70 | 0.38 | 3.7 | 484.7 | 4.12 |
| 6 | 0.39 | 4.7 | 0.42 | 22.4 | 6.28 | 0.44 | 5.3 | 533.2 | 4.53 |
| 7 | 0.44 | 5.3 | 0.47 | 23.1 | 6.47 | 0.49 | 5.2 | 482.8 | 4.11 |
| 8 | 0.49 | 5.4 | 0.52 | 17.1 | 4.79 | 0.54 | 2.3 | 379.3 | 3.23 |
| 9 | 0.54 | 2.6 | 0.58 | 65.9 | 18.43 | 0.62 | 1.2 | 1753.1 | 14.91 |
| 10 | 0.68 | 6.2 | 0.70 | 11.8 | 3.30 | 0.75 | 0.2 | 329.8 | 2.80 |
| 11 | 0.83 | 3.6 | 0.94 | 117.7 | 32.92 | 0.99 | 17.0 | 6138.1 | 52.20 |
| 12 | 0.99 | 3.6 | 0.94 | 18.8 | 5.25 | 1.00 | 17.0 | 82.0 | 0.70 |




| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.0 | 0.01 | 106.0 | 52.16 | 0.08 | 5.8 | 2314.7 | 49.43 |
| 2 | 0.72 | 0.9 | 0.73 | 56.3 | 27.71 | 0.75 | 0.4 | 307.2 | 6.56 |
| 3 | 0.82 | 3.4 | 0.90 | 26.3 | 12.92 | 0.95 | 13.4 | 1650.6 | 35.25 |
| 4 | 0.95 | 13.6 | 0.95 | 14.6 | 7.21 | 0.99 | 4.1 | 409.9 | 8.75 |

**Figure 10 HPTLC finger print profile of chloroform extract of Gmelina asiatica L fruit pulp a) UV 254 nm. b) UV 354 nm.**




| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.33 | 1.2 | 0.38 | 85.1 | 30.83 | 0.41 | 7.4 | 2283.6 | 30.08 |
| 2 | 0.42 | 7.5 | 0.45 | 20.8 | 7.55 | 0.51 | 0.9 | 816.3 | 10.75 |
| 3 | 0.55 | 0.1 | 0.59 | 56.2 | 20.35 | 0.64 | 0.1 | 1426.5 | 18.79 |
| 4 | 0.88 | 1.4 | 0.92 | 38.3 | 13.87 | 0.94 | 32.6 | 1100.0 | 14.49 |
| 5 | 0.95 | 32.7 | 0.98 | 75.7 | 27.40 | 1.00 | 4.0 | 1965.1 | 25.88 |

| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.07 | 0.4 | 0.09 | 16.8 | 33.28 | 0.12 | 0.1 | 280.8 | 17.63 |
| 2 | 0.83 | 4.6 | 0.91 | 33.6 | 66.72 | 0.94 | 1.4 | 1311.3 | 82.37 |

| Peak | Start Position (Rf) | Start Height (AU) | Max Position (Rf) | Max Height (AU) | Max % | End Position (Rf) | End Height (AU) | Area (AU) | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 56.0 | 0.01 | 58.3 | 24.82 | 0.03 | 0.4 | 540.7 | 10.90 |
| 2 | 0.04 | 1.1 | 0.06 | 53.9 | 22.95 | 0.07 | 0.1 | 662.3 | 13.35 |
| 3 | 0.15 | 0.2 | 0.17 | 12.6 | 5.37 | 0.19 | 2.5 | 200.0 | 4.03 |
| 4 | 0.35 | 2.3 | 0.39 | 15.7 | 6.67 | 0.41 | 7.3 | 429.1 | 8.65 |
| 5 | 0.41 | 6.9 | 0.44 | 22.8 | 9.70 | 0.46 | 9.1 | 525.2 | 10.59 |
| 6 | 0.46 | 9.3 | 0.48 | 24.0 | 10.21 | 0.51 | 15.2 | 683.2 | 13.77 |
| 7 | 0.51 | 14.9 | 0.54 | 25.7 | 10.94 | 0.58 | 14.7 | 1029.7 | 20.75 |
| 8 | 0.75 | 7.1 | 0.79 | 21.9 | 9.34 | 0.83 | 5.9 | 891.3 | 17.97 |

**Figure 11. HPTLC finger print profile of ethanolic extract of Gmelina asiatica fruit pulp. a) UV 254 nm. b) UV 354 nm. c) long white light 520nm.**

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**RESEARCH ARTICLE**

# Microbial Derived Vitamin D2 and Nisin as Dual Function Compounds with Antibiofilm and Anticancerous Activities

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## ABSTRACT

This study was conducted to investigate the potential of microbial- derived commercially available Vitamin D2 and Nisin as dual function compounds with antibiofilm and anticancerous properties; exploring their mechanism of action and therapeutic implications. Utilizing a combination of cultures, comprehensive array of laboratory experiments and analytical tools, precise and robust analysis was carried out. The compounds Vitamin D2 and Nisin were subjected to rigorous testing against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial strains along with A431 SCC cancer cell lines to assess their antibiofilm and anti-cancerous activity respectively. The results demonstrated that microbial- derived Vitamin D2 and Nisin exhibit potential antibacterial effects. Additionally, both compounds also show promising anti-cancerous activity, hindering the proliferation of cancer cells with notable specificity in MTT and Apoptosis assay. This research establishes microbial- derived Vitamin D2 and Nisin as promising dual- action compounds with potential applications in both antibacterial therapies and anticancer treatments, highlighting their multifaceted therapeutic significance.

**Keywords:** Nisin, Vitamin D2, apoptosis, MTT Assay, A431 SCC cancer cell lines.

## INTRODUCTION

The world is on the verge of entering the "post-antibiotic era," a time when the mortality rate from common bacterial infections will surpass that of cancer [1]. Multidrug resistance is one of the most significant global public health threats of the twenty-first century and may also be related to antimicrobial medications. Because of treatment failures and their prevalence in healthcare costs, this phenomenon has indeed increased both mortality and morbidity [2]. Gram-positive and gram-negative bacteria with multidrug resistance have led to infections that are difficult to treat

or even untreatable with conventional antimicrobials. Additionally, biofilms can be problematic for infection control because they contribute to multidrug resistance. Since biofilms permit microbial cells to briefly enter a metabolically inactive state, antibiotics are rendered ineffective, which leads to disease recurrence because latent bacterial infection is still present within the host [3]. High antibiotic doses for an extended period are typically necessary to combat this cell organization, but these methods frequently fall short, resulting in infection persistence [4]. The treatment and control of cancer is also a major cause of concern for both developing and developed nations. Conventional anti-cancer therapy involves the use of chemical chemotherapeutics and radiation and is often non-specific in action. The development of drug resistance and the inability of the drug to penetrate the tumor cells has been a major pitfall in current treatment.

The creation of novel, "out of the box," therapeutics is required to combat antibiotic-resistant bacterial infections brought on by acquired resistance and/or biofilm formation and the need to investigate alternative anti-tumor therapeutics possessing greater specificity and efficacy is significant [5-6]. Some of the microorganisms produce bioactive secondary metabolites that may be involved in a host-endophyte relationship. Recently, many endophytic bioactive molecules, known as well as new substances, possessing a wide variety of biological activities such as antibiotic, antitumor, anti-inflammatory, antioxidant, etc. have been identified among which Vitamin D2 and Nisin will be assessed in this study.The anti-bacterial and anti-quorum sensing activity of vitamin D2 from fungus is evident from mushrooms but from yeasts remains unexplored. Also, there are restricted resources regarding the anticancerous activity of commercially available vitamin D2 from yeasts. The use of nisin as a single agent or in combination with other conventional medications to treat cancer is still in its infancy. The microbiome and cancer may also be related etiologically, according to mounting evidence.

## MATERIALS AND METHODS

**Procurement of Samples, Strains& Cell Cultures**

Vitamin D2 (S1) and Nisin (S2) were procured from I. A Chemicals, Gujarat and Chihonbio respectively of 10 grams. Atotal of two microorganisms were used to assess the antimicrobial properties, *Staphylococcus aureus* (MTCC-2408), and *Pseudomonas aeruginosa (*MTCC 2080).A431 (Human skin cancer) cell line was initially procured from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained Dulbecco's modified Eagles medium, DMEM (Sigma-Aldrich, USA).

**Antibiofilm Activity by Crystal violet Assay Method**

**Sample preparation**

Test samples Vitamin D2 & Nisin were dissolved in 0.02 N HCl & Methanol respectively at a concentration of 100 mg/mL. This was further diluted in LB broth to obtain test concentrations of 0.0097, 0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5 & 5 mg/mL. Similar dilutions were prepared for standard Gentamicin. The protocol was laid as per the method by O'Toole**[7].**

**Anti-Cancerous Potency by MTT and Apoptosis Assays on A431 cell lines**

The cell line was cultured in a 25 $cm^2$ tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany), and an antibiotic solution containing: Penicillin (100 μg/ml), Streptomycin (100 μg /ml), and Amphotericin B (2.5 μg/ml). Cultured cell lines were kept at $37^0$C in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by an Inverted phase contrast microscope and followed by the MTT assay method.

**Anticancer Evaluation**

After 24 hours the growth medium was removed, and freshly prepared compounds in DMEM were five times serially diluted by two-fold dilution (200 µg, 100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 500 µl of DMEM) and each concentration of 100 µl was added in triplicates to the respective wells and incubated at $37^0$C in a humidified 5% $CO_2$ incubator. Non-treated control cells were also maintained[**8**].

The percentage of growth inhibition was calculated using the formula:

% of viability = Mean of OD samples / Mean of OD Control x 100

**Apoptosis Assay**

After treatment with the sample at LC 50 Concentration (S1-114.29 µg/mL and S2 -137.142 µg/mL) for 24 hours, the cells were washed with cold PBS and then stained with a mixture of AO (100 µg/ml) and EtBr (100 µg/ml) at room temperature for 10 min. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in the blue filter of the fluorescent microscope (Olympus CKX41 with Optika Pro5 camera)[9].

**STATISTICAL ANALYSIS**

All the experiments were performed in triplicates and the results are expressed as Mean ± SD (n = 3). The results were analyzed for statistical significance using the unpaired Students T-test, One-way ANOVA, and Dunnets test (SPSS Inc. 20.0 version) & ED50 PLUS V1.0 Software. Probability values (P) ≤ 0.05 were statistically significant.

## RESULTS

**Antibiofilm Activity by Crystal Violet Assay Method**

The biofilm inhibition activityof Vitamin D2 and Nisin was performedagainst thetestmicroorganisms namely *S. aureus*&*P. aeruginosa*. Gentamicin was used as the positive control. 10 test concentrations of the samples were taken from 5- 0.0097 mg/ml. Vitamin D2 depicted more inhibition activity compared to Nisin. The IC50 values of Vitamin D2, Nisin, and Gentamicin were 0.080 mg/ml, 1.231 mg/ml, and 0.028 mg/ml respectively against *S. aureus* whereas IC50 values of Vitamin D2, Nisin, and Gentamicin were 0.202 mg/ml, 1.453 mg/ml, and 0.037 mg/ml respectively against *P. aeruginosa*.The results are tabulated in Tables 1A &1B. The comparative analysis of the activity is illustrated in Figures 1A& 1B.

**Anticancerous Potency by MTT Assay Method**

The test samples Vitamin D2 & Nisin were assessed for their anticancerous potency on human skin cancer cell lines-A431 cell lines. Non-treated cells were used as control. Indicators of cytotoxicity like granulation, rounding, and vacuolization were observed under microscopic examination. The percentage of growth inhibition was also calculated wherein the expressed LC50 Values of Vitamin D2 and Nisin were 114.29 µg/mL and 137.14 µg/mLrespectively (Calculated using ED50 PLUS V1.0 Software). The percentage of viability and cytotoxicity are represented in Figures 2A, 2B, 2C, and2D respectively.

**Anticancerous Potency by Apoptosis Assay Method**

A431 cell lines appeared to exhibit apoptosis on treatment with test samples Vitamin D2 & Nisin. Non-treated cells were used as control. The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation), and necrotic cells (uniformly orange-stained cell nuclei) which are depicted in Figure 3.

## DISCUSSION AND CONCLUSION

Along with the struggle to combat AMR, cancer is also a complex and devastating disease that causes a range of health problems. Therefore, a general call for novel and therapeutic naturally occurring metabolites to render or treat infections and diseases points out to be a significant highly effective alternative. In this sense, the antimicrobial and anticancerous activity of the metabolite Vitamin D2 and lantibiotic Nisin was analyzed against microbes and skin cancer SCC cell lines respectively. In the initial screening of antibiofilm activity of the samples, the Crystal violet assay method depicted that both Vitamin D2 and Nisin can inhibit the biofilm formation of the test organisms *S. aureus* and *P. aeruginosa.* This opens a new strategy of using these compounds to resist biofilm formation that has been exacerbating infections. The test samples were taken at a concentration ranging from 5- 0.0097 mg/ml. The IC50 values of Vitamin D2, Nisin, and Gentamicin (positive control) were 0.080 mg/ml, 1.231 mg/ml, and 0.028 mg/ml respectively against *S. aureus* whereas IC50 values of Vitamin D2, Nisin, and Gentamicin were 0.202 mg/ml, 1.453 mg/ml, and 0.037 mg/ml respectively against *P. aeruginosa*.

The anticancerous potency of Vitamin D2 & Nisin analyzed by MTT & Apoptosis assay on Human skin cancer SCC A431 cell lines also proved that these compounds can be used as an alternative to treat skin cancers instead of conventional chemotherapeutic methods which cause severe side effects. Moreover, since Vitamin D2 is non-calcaemic, it can be replaced instead of Vitamin D3. In MTT assay indicators of cytotoxicity like granulation, vacuolation, and rounding were observed. The percentage of growth inhibition was also calculated wherein the expressed LC50 Values of Vitamin D2 and Nisin were 114.29 μg/mL and 137.14 μg/mL respectively. In the Apoptosis assay, cells exhibiting apoptosis were observed under an inverted phase contrast microscope. This work could help in groundbreaking medical interventions that address infection control and cancer treatment challenges simultaneously. The future perspective of this study paves the way for analyzing the action of Nisin and Vit D2 in the gene regulation responsible for biofilm inhibition at the molecular level. Furthermore, by docking methods, the extent of activity by Vitamin D2 and Nisin on targeted cells could also be analyzed.

## DECLARATIONS

## ACKNOWLEDGEMENT

I acknowledge the Department of Microbiology, Krupanidhi Degree College wherein I have carried out the current work. My sincere gratitude to Biogenix Research Lab wherein I have carried out the cancer studies

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There are no funding bodies in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**CONFLICT OF INTEREST**
The authors "declare that they have no conflict of interest."

**Ethics Approval and Consent to Participate**
"This article does not contain any studies with human participants or animals performed by any of the authors."

**Consent for Publication**
This manuscript does not contain any personal data of individuals in any forms.

**Availability of Data and Material**
"Data sharing not applicable to this article as no datasets were generated or analysed during the current study".

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**Table 1A.0020AntibiofilmActivityof Vitamin D2 & Nisin against *Staphylococcus aureus***

| Crystal violet OD @ 595 nm | | | Test Concentrations (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Blank** | **Untreated** | **0.0097** | **0.019** | **0.039** | **0.078** | **0.156** | **0.312** | **0.625** | **1.25** | **2.5** | **5** |
| **Gentamicin (Positive control)** | | | | | | | | | | | | |
| **Mean OD ± SD** | 0.06 | 1.5 ± 0.1 | 1.42 ± 0 | 1.25 ± 0.03 | 0.2 ± 0 | 0.05 ± 0.02 | 0.05 ± 0 | 0.06 ± 0.03 | 0.04 ± 0 | 0.05 ± 0 | 0.06 ± 0.01 | 0.05 ± 0.0 |
| **% of Biofilm Inhibition** | | 0 | 5.3 | 16.6 | 86.6 | 96.7 | 96.7 | 96 | 97.3 | 96.7 | 96 | 96.7 |
| **IC50= 0.028 mg/mL** | | | | | | | | | | | | |
| **Vitamin D2 (S1)** | | | | | | | | | | | | |
| **Mean OD ± SD** | 0.06 | 1.5 ± 0.1 | 1.53 ± 0.12 | 1.44 ± 0.07 | 1.5 ± 0.14 | 0.8 ± 0.11 | 0.2 ± 0.06 | 0.06 ± 0.02 | 0.06 ± 0.0 | 0.07 ± 0.02 | 0.05 ± 0.01 | 0.06 ± 0.02 |
| **% of Biofilm Inhibition** | | 0 | -2 | 4 | 0 | 46.7 | 86.7 | 96 | 96 | 95.3 | 96.7 | 96 |
| **IC50= 0.080 mg/mL** | | | | | | | | | | | | |
| **Nisin (S2)** | | | | | | | | | | | | |
| **Mean OD ± SD** | 0.06 | 1.5 ± 0.1 | 1.6 ± 0.04 | 1.6 ± 0.08 | 1.6 ± 0.1 | 1.6 ± 0.02 | 1.43 ± 0 | 1.52 ± 0.04 | 1.6 ± 0.03 | 0.51 ± 0.02 | 0.2 ± 0.0 | 0.04 ± 0.01 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | 2 | |
| **% of Biofilm Inhibition** | | 0 | -6.67 | -6.67 | -6.67 | -6.67 | 4.6 | -1.3 | -6.67 | 66 | 86.7 | 97.3 |
| **IC50= 1.231 mg/mL** | | | | | | | | | | | | |

**Table 1B**. AntibiofilmActivityof Vitamin D2 & Nisin against *Pseudomonas aeruginosa*

| **Crystal violet OD @ 595 nm** | | | **Test Concentrations (mg/ml)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Blank** | **Untreated** | **0.0097** | **0.019** | **0.039** | **0.078** | **0.156** | **0.312** | **0.625** | **1.25** | **2.5** | **5** |
| **Gentamicin (Positive control)** | | | | | | | | | | | | |
| **Mean OD ± SD** | 0.06 | 1.41 ± 0.03 | 1.35 ± 0.05 | 1.41 ± 0.05 | 0.5± 0.07 | 0.2± 0.05 | 0.09± 0.02 | 0.07 ± 0 | 0.04 ± 0.02 | 0.06 ± 0 | 0.05 ± 0.03 | 0.07 ± 0 |
| **% of Biofilm Inhibition** | | 0 | 4.2 | 0 | 64.5 | 85.8 | 93.6 | 95 | 97.1 | 95.7 | 96.4 | 95 |
| **IC50= 0.037 mg/mL** | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vitamin D2 (S1)** | | | | | | | | | | | | |
| **Mean OD ± SD** | 0.06 | 1.5 ± 0.1 | 1.53 ± 0.12 | 1.44 ± 0.07 | 1.5 ± 0.14 | 0.8 ± 0.11 | 0.2 ± 0.06 | 0.06 ± 0.02 | 0.06 ± 0.0 | 0.07 ± 0.02 | 0.05 ± 0.01 | 0.06 ± 0.02 |
| **% of Biofilm Inhibition** | | 0 | -2 | 4 | 0 | 46.7 | 86.7 | 96 | 96 | 95.3 | 96.7 | 96 |
| **IC50= 0.202 mg/mL** | | | | | | | | | | | | |
| **Nisin (S2)** | | | | | | | | | | | | |
| **Mean OD ± SD** | 0.06 | 1.41 ± 0.03 | 1.6 ± 0.07 | 1.62 ± 0.09 | 1.6 ± 0.07 | 1.4 ± 0.02 | 1.4 ± 0.03 | 1.33 ± 0.01 | 1.34 ± 0.05 | 1.21 ± 0.08 | 0.13 ± 0.01 | 0.06 ± 0 |
| **% of Biofilm Inhibition** | | 0 | -13.5 | -14.9 | -13.5 | 0.71 | 0.71 | 5.7 | 5.0 | 14.2 | 90.8 | 95.7 |
| **IC50= 1.453 mg/mL** | | | | | | | | | | | | |

Data shown are the average and standard deviation based on duplicate runs (Mean ± StandardDeviation)

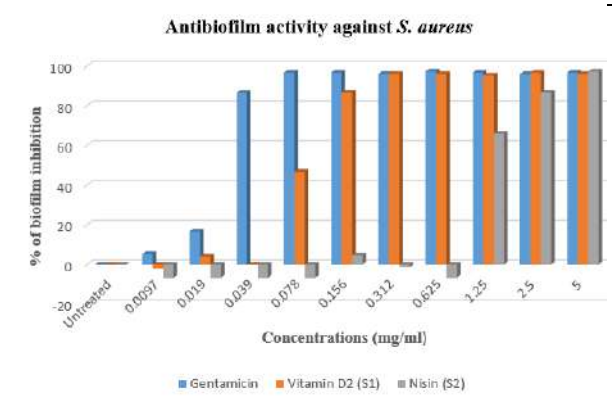


**Figure 1A: Antibiofilm activity of Vitamin D2 & Nisin against *Staphylococcus aureus***

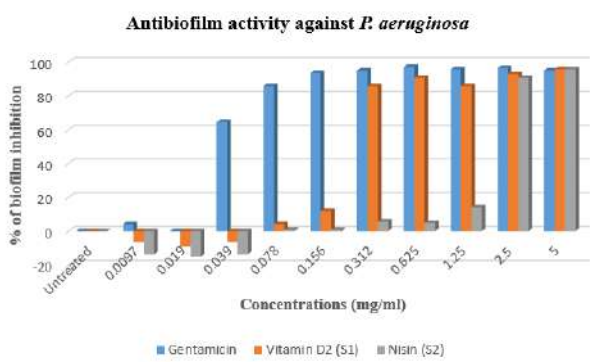


**Figure 1B: Antibiofilm activity of Vitamin D2 & Nisin against *P. aeruginosa***

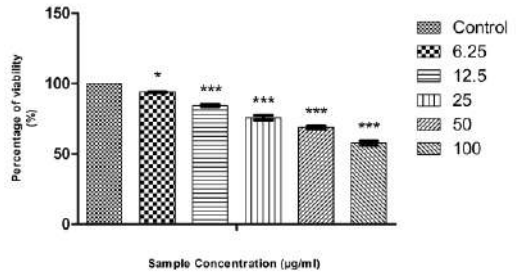


**Figure 2A: Graphical representation depicting the anticancer effect of Vitamin D2 (S1) on cell line by MTT assay. Along the Y axis Percentage viability, Along the X axis varied concentration of S1. All experiments were done in triplicates and results were represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyze data. ***p < 0.001 compared to control groups, **p < 0.01 compared to control groups, *p < 0.1 compared to control groups.**

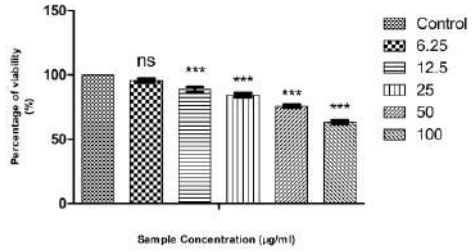


**Figure 2B: Graphical representation depicting the anticancer effect of Nisin (S2) on A431cell line by MTT assay. Along the Y axis Percentage viability, Along the X axis varied concentrations of S2. All experiments were done in triplicates and results were represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyze data. ***p < 0.001 compared to control groups, **p < 0.01 compared to control groups, *p < 0.1 compared to control groups.**

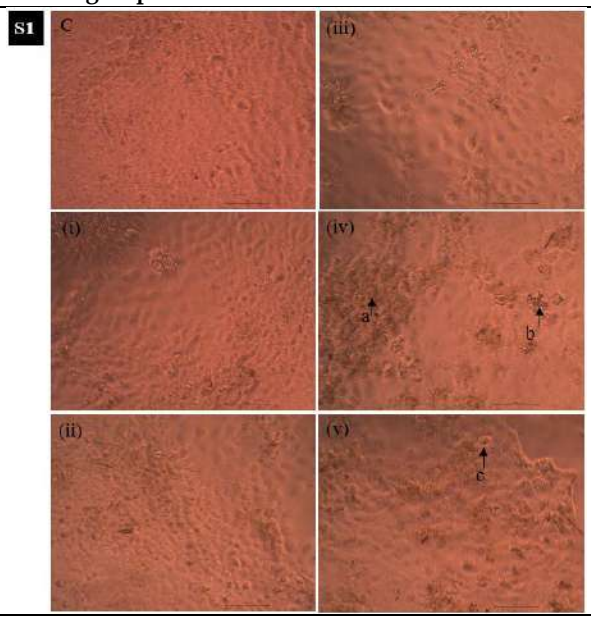


**Figure 2C.Microscopic examination of cytotoxicity of Vitamin D2 on A431 cell lines under 40X magnification in inverted phase contrast microscope. [C- Untreated control cells exhibiting epithelial morphology; (i)- 6.25 µg/ml; (ii)- 12.5 µg/ml; (iii)- 25 µg/ml; (iv)- 50 µg/ml; (v)- 100 µg/ml; a- granulation; b- vacuolization; c- rounding which is considered as a sign of cytotoxicity].**

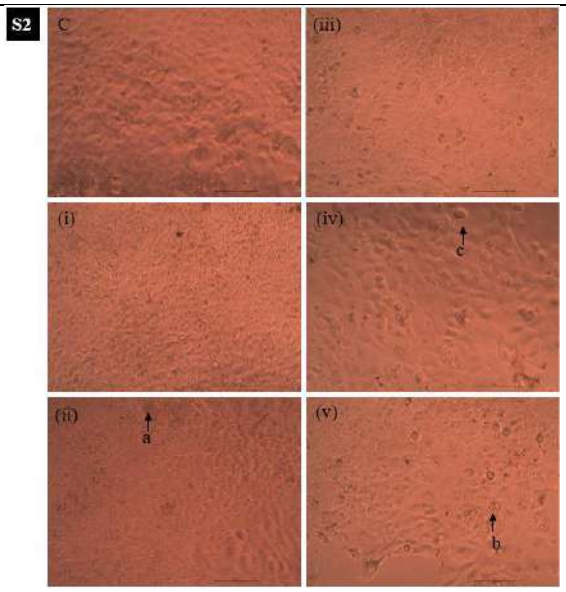


**Figure 2D: Microscopic examination of cytotoxicity of Nisin on A431 cell lines under 40X magnification in inverted phase contrast microscope. [C- Untreated control cells exhibiting epithelial morphology; (i)- 6.25 µg/ml; (ii)- 12.5 µg/ml; (iii)- 25 µg/ml; (iv)- 50 µg/ml; (v)- 100 µg/ml; a- granulation; b- vacuolization; c- rounding which is considered as a sign of cytotoxicity]**

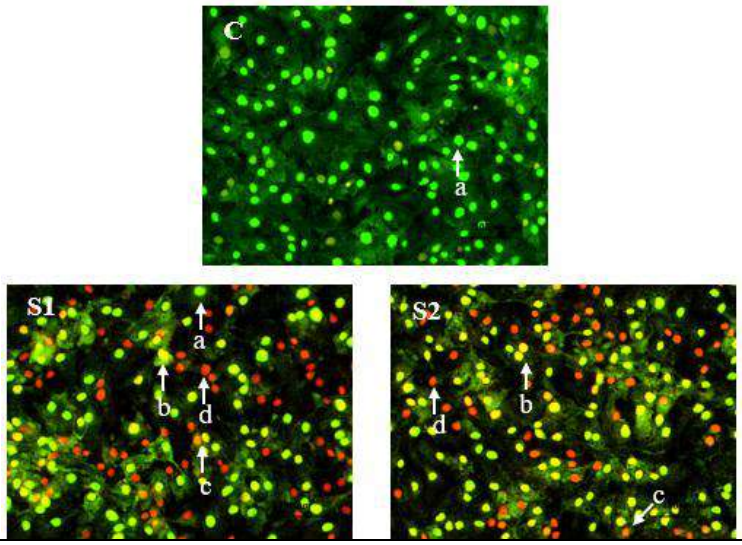


**Figure 3.Apoptosis of cells on treatment with Vitamin D2 & Nisin observed in a fluorescent microscope**
**[S1- Vitamin D2; S2- Nisin; C- Control; a- Living cells; b- early apoptotic cells; c- late apoptotic cells; d- necrotic cells].**

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**RESEARCH ARTICLE**

# Efficacy of Kiastm along with Motor Control Training Exercises in Chronic Non-Specific Neck Pain Patients - A Pilot Study

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## ABSTRACT

Neck pain has been referred to as "nonspecific" or "mechanical" neck pain since the great majority of cases do not result from organic pathology. KIASTM ("Kinesio Instrument Assisted Soft Tissue Mobilization") is a method in which soft tissue components are mechanically stimulated by tools to reduce musculoskeletal pain and discomfort and enhance mobility and function. Generally, it explains the use of an Accel instrument, which is ergonomically designed to help doctors identify and treat soft tissue pain, injury, and dysfunction. Numerous prospective analyses have revealed that people with chronic neck pain may also have weak neck muscles and motor control problems. The MCE (Motor Control Exercise) is a treatment technique that primarily focuses on motor control, and activation of deep cervical muscles, as well as aims to retrain the best control & coordination of the cervical muscles. The objective of this research was to check the effectiveness of Motor control training exercises and KIASTM in Chronic nonspecific neck pain patients in terms of pain, soft tissue mobility, and functional disability. On the basis of inclusion criteria, 5 subjects were selected and baseline data were collected on day one for Pain using VAS (Visual Analogue Scale), Universal Goniometer for Cervical range of motion, DNF (Deep Neck Flexor) Endurance Testing for deep neck flexors, and functional disability using NDI (Neck Disability Index). Interventions applied include Transcutaneous Electrical Nerve Stimulation for 15 minutes followed by application of KIASTM for 90 seconds as per protocol and Motor control training exercises for 3 sessions per week. The total duration of treatment was 3 weeks.After 3 weeks of interventions, the results showed significant improvement in terms of pain, ROM, DNF Endurance level, and disability following applications of KIASTM along with the Motor Control Exercise program in Chronic nonspecific neck pain cases.A considerable amount of improvement was observed following the application of KIASTM along

with other therapeutic interventions like TENS and MCEsin reducing pain, increasing AROM, improvement in function, and deep neck flexor endurance during the rehabilitation of chronic nonspecific neck pain patients.

**Keywords**: Nonspecific neck pain, IASTM, Motor Control exercise, Physiotherapy.

## INTRODUCTION

Neck pain is among the most frequent and serious musculoskeletal conditions. Point prevalence range between 6 to 22 percent, and in the elderly population, it may reach up to 38 percent, whereas lifetime prevalence varies from 14.2 to 71 percent [1]. These are assumed to be generalized neck pain. Nonspecific neck pain is just neck pain without a known underlying illness as the source of the pain. Symptoms change with physical exercise and with time. Non-specific neck pain refers to any kind of chronic neck pain, subacute, or acute when no abnormal anatomic feature could be recognized as the source of the pain. There are several viewpoints about the symptom's duration but as per Binder, neck pain may be acute (less than 4 weeks duration), sub-acute (1-4 months duration), or chronic (more than 4 months duration). Chronic neck pain may result in significant medical expenses, job absences, and disability.Depending on the length, neck pain may affect functional ability and quality of life, in addition to causing stress, depression, and anxiety [2]. Therefore, neck pain exerts a significant burden on people, employers, as well as healthcare systems. Existing literature explored many diverse treatment approaches for nonspecific neck pain. As the majority of the patients with nonspecific neck pain have nondefinitive pathology, hereby fail to direct treatment. Many patients get conservative care from a general physiotherapist or a physician but there is always a lack of proper indications of the conditions for any specific management. The manual treatment method recognized as IASTM ("Instrument-Assisted Soft-Tissue Mobilization") locates and treats soft tissue problems by using rigid tools of different forms and materials. IASTM is a non-invasive treatment method that is often used by stroking the edge of a tool on the skin's surface, sometimes with the help of lubricant, with the goal of affecting the underlying connective tissues, muscles, as well as nerves [3]. As such, many IASTM instruments, companies, and proposed application protocols. IASTM is an umbrella term.Various types of brands are available. Various types include KIASTM, Sound Assisted Soft tissue mobilization, Gua Sha, Graston Technique, Fascial Abrasion Technique, and Augmented Soft Tissue Mobilization Technique. KIASTM is a process where soft tissue components are mechanically stimulated with the use of devices to reduce musculoskeletal pain and discomfort and enhance general mobility and functional capacity. It often refers to using an accel tool. Accel tool, which is ergonomically created to help professionals identify and treat soft tissue pain, damage, and dysfunction. The KIASTM method also incorporates a standard protocol to be followed while applying the technique in various musculoskeletal and neurological conditions with the involvement of myofascia. It includes the following techniques: scanning to check for limits across the affected region, combining with deeper, multidirectional strokes, and gliding to provide a cool-down period.

According to prospective research, those who suffer from persistent neck discomfort have weak neck muscles and reduced motor function. The therapy method that primarily focuses on motor control, and deep cervical muscles activation, and seeks to retrain the cervical muscles' ideal control and coordination is known as motor control exercises. It has been demonstrated that the MCE, which is typically formed under supervision, improves motor control while decreasing pain and impairment in individuals with neck discomfort. The exercise focuses on the deep flexor muscles of the upper cervical region, the longus capitis and longus colli muscles, as opposed to the superficial flexor muscles, the sternocleidomastoid and anterior scalene, which flex the neck but not the head [4]. The motor control is characterized as a motor relearning program that places a focus on the coordination and holding capacities of certain neck flexors, extensors, and shoulder girdle muscles. As there is a lack of enough supporting literature regarding the usage of IASTM in chronic nonspecific neck pain and also addressing the weakness of deep neck flexors in the same condition, the management of this condition becomes challenging for the therapist. Chronic nonspecific neck pain has become alarming for the global population due to workload demand, excessive usage of electronic gadgets, and the sedentary lifestyle of the present generation. Weakness of deep neck flexors will lead to neuromuscular incoordination and ultimately failure of a rehabilitation program. So addressing the pain, fascia, and

muscle tightness with the help of Kinesio Instrument Assisted Soft Tissue Mobilization along with Motor control Training exercises was the prime objective of this pilot study. Thereby the need for this pilot study to check the effectiveness of KIASTM along with the Motor Control Training Exercise program in chronic nonspecific neck pain.

## METHODOLOGY

**Study Design**

A group of 6 patients participated in the single-group experimental pilot study after fulfilling the inclusion as well as exclusion criteria. Participants have been assessed based on the level of pain with VAS, range of motion (ROM) using goniometry, DNF Endurance testing, and disability score using Neck Disability Index during the preintervention and postintervention phases. The pilot research was conducted for 3 weeks. Before the research started, each of the selected individuals signed an informed permission form in line with the Helsinki Rules and expressed their intention to take part in the study.

**Study Population**

Calculation of the sample size was done after a survey was performed where it has been found that the population proportion of patients reported with nonspecific neck pain is 1.1%. The required size of the sample was computed with Cochran's formula for infinite population keeping a confidence interval of 99.9%, margin of error 5%, and population proportion 1.1%. Participants of both sexes, complaining of persistent nonspecific neck pain, and falling within the age range of 18 to 40 years were required for participation in the research. On the other hand, individuals who had any neck injuries in the three months previous to the trial had spine surgery during the preceding six months, missed more than 15 percent of the intervention sessions (2 sessions), and did not sign the informed consent form were excluded from the study.

**Procedure**

Physiotherapy interventions were given with a frequency of three sessions every week for a duration of 3 weeks. The interventions were applied after fulfilling the inclusion criteria by the patient and after collecting informed consent. The therapy sessions included the application of TENS, KIASTM, and Motor Control Training exercise protocol. TENS was given for 15 minutes for each patient followed by the application of KIASTM for 90 seconds using Accel Tool with a three-step process starting with scanning of the affected area in the neck region primarily focusing the upper trapezius muscle fibers on both sides, then application of the tool with little deeper pressure to release the restricted soft tissue and lastly by stroking the muscle fiber with light pressure as a cool-down phase for winding up the treatment. After the application of KIASTM, patients were asked to complete the Motor Control Training Exercise protocol (Griffith et al.) [6] with proper instructions from the therapist. The subjects have been also asked to keep the activity of the deep neck flexor group of muscles by incorporating them into their daily functional activities.

Four dependent variables were evaluated: pain using Visual Analogue Scale (VAS), range of motion for cervical extension, lateral flexion, flexion, as well as rotation by a universal goniometer, muscle strength of deep neck flexors by using DNF Endurance testing, and lastly functional level by Neck Disability Index (NDI) pre-intervention & post intervention. Level of pain was evaluated by VAS which comprises a line, often 10cm long, with verbal anchors at either end, similar to the Numerical Rating Scale depicted as "no pain" on the far left and "the most intense pain imaginable" on the far right. The patient marks the point on the line that corresponds to their assessment of the level of pain.

The Harris et al. approach was used for conducting the DNF muscular endurance test for this investigation . The test was carried out in a hook-lying, supine posture. The participant was expected to flex the chin to its maximum extent and maintain the chin tuck posture for the duration of the test. They were then instructed to elevate their heads until they were about 2.5 cm above the plinth while retaining a chin tuck. Once the participant was in place, a line was drawn across their two approximate neck skin folds. On the surface of the table, right below the participant's occiput,

the examiner positioned his cephalad hand. When the line edges started to split or the participant's head contacted the therapist's hand, further verbal directions were provided. The test was stopped if the participant's head contacted the therapist's hand for more than a second or if the lines on the chart were no longer approximated owing to loss of the chin tuck posture [7].

## RESULTS

The current research was intended to check the combined role of the effectiveness of KIASTM & Motor Control Training Exercises along with conventional physiotherapy in chronic nonspecific neck pain patients. The pre-intervention VAS Mean score was 7.4 with a "Standard Deviation" (SD) of 0.548. The correlation between the pre-intervention VAS scores and post-intervention scores was 0.667. The t-test comparing pre- and post-intervention VAS scores showed a significant difference with a high t-value of 11.18 ($p < 0.001$). Post Intervention VAS Mean score was 2.4, indicating a significant improvement from the pre-intervention score. Pre-Intervention DNS (Deep Neck Strength) testing Mean score was 12.618 with a SD of 4.47405. The correlation between the pre-intervention DNS scores and post-intervention DNS scores was 0.855. The t-test comparing pre- and post-intervention DNS scores showed a significant difference with a t-value of -7.289 ($p = 0.002$).

Pre-Intervention NDI (Neck Disability Index) Mean score was 39.94 with a SD of 5.1247. The relationship between the pre-intervention NDI scores and the post-intervention NDI scores was 0.612. The t-test comparing pre- and post-intervention NDI scores showed a significant difference with a t-value of 12.010 ($p < 0.001$). Post Intervention NDI Mean score was 14.7, indicating a significant improvement from the pre-intervention score. Pre-Intervention CF (Cervical Flexion) Mean score was 47.2 with a SD of 5.119. The relationship between the pre-intervention CF scores and the post-intervention CF scores was -0.273. The t-test comparing pre- and post-intervention CF scores showed a substantial difference with a t-value of -3.860 ($p = 0.018$). Post Intervention CF Mean score was 57.6, suggesting an improvement in cervical flexion compared to the pre-intervention scores.

Pre-Intervention CE (Cervical Extension) Mean score was 59.6 with a SD of 9.397. The correlation between the pre-intervention CE scores and the post-intervention CE scores was 0.754. The t-test comparing pre- and post-intervention CE scores showed a significant difference with a t-value of -3.469 ($p = 0.026$). Post Intervention CE Mean score was 69.2, indicating an improvement in cervical extension compared to the pre-intervention scores. Pre-Intervention RLF (Right Lateral Flexion) Mean score was 44 with a standard deviation of 2.646. The correlation between the pre-intervention RLF scores and the post-intervention RLF scores was 0.125. The t-test comparing pre- and post-intervention RLF scores showed a significant difference with a t-value of -3.570 ($p = 0.023$). Post Intervention RLF Mean score was 48.6, suggesting an improvement in right lateral flexion compared to the pre-intervention scores.

Pre-Intervention LLF (Left Lateral Flexion) Mean score was 36.6 with a SD of 4.775. The relationship between the pre-intervention LLF scores and the post-intervention LLF scores was -0.255. The t-test comparing pre- and post-intervention LLF scores did not reach statistical significance with a t-value of -2.211 ($p = 0.091$). Post Intervention LLF Mean score was 44.4, suggesting an improvement in left lateral flexion compared to the pre-intervention scores. Pre-Intervention RLR (Right Lateral Rotation) Mean score was 54 with a SD of 7.517. The relationship between the pre-intervention RLR scores and the post-intervention RLR scores was 0.805. The t-test comparing pre- and post-intervention RLR scores did not reach statistical significance with a t-value of -2.092 ($p = 0.105$). Post Intervention RLR Mean score was 59.4, indicating an improvement in right lateral rotation compared to the pre-intervention scores.

Pre-Intervention LLR (Left Lateral Rotation) Mean score was 54.2 with a SD of 2.588. The relationship between the pre-intervention LLR scores and the post-intervention LLR scores was -0.382. The t-test comparing pre and post-intervention LLR scores did not reach statistical significance with a t-value of -2.409 ($p = 0.074$). Post Intervention LLR

Mean score was 63, suggesting an improvement in left lateral rotation compared to the pre-intervention scores. In summary, the overall intervention showed statistically significant improvements in VAS, DNS, NDI, CF, CE, RLF, and LLF scores. However, post-intervention CF, RLR, and LLR scores indicated higher levels of disability or limited improvement compared to the pre-intervention scores.

## DISCUSSION

The proposed physical therapy protocol indicated substantial enhancement in terms of neck pain reduction and an increase in deep neck muscle endurance and functional level in patients having chronic nonspecific neck pain. These findings have contributed to a few earlier pieces of literature where instrument-aided soft tissue mobilization and motor control training exercises for deep neck muscles have shown positive outcomes in separate studies for chronic nonspecific neck pain cases. The present study also has a few limitations like a small sample size and only female participants for which the findings cannot be generalized for everyone. More number of participants including both genders could be considered for future studies. The present pilot study showed a reduction of pain and an increase in the cervical range of motions in all directions which is contributing to previous studies where the use of IASTM and neuromuscular exercises have shown better results in terms of forward head position and functioning (Mylonas K et al.) [8]. Other studies have also demonstrated the immediate effect of IASTM for the reduction of pain in chronic nonspecific neck pain patients along with correction of joint position error (Gereck H et al.) [9]. The impact of Motor Control Training Exercises was demonstrated in very limited literature for neck pain. The role of Motor Control Training Exercises in improving the strength of deep neck muscles has been demonstrated in the present pilot study while calculating the DNS testing results for each participant. Therefore, incorporating exercises for activating deep neck muscles even during functional activities of daily life will have a significant impact in maintaining and improvement of deep neck muscle endurance thereby reduction of neck disability in patients having chronic nonspecific neck pain. These results are contributing to similar findings in previous studies in the form of systematic review and metanalysis conducted where incorporating Motor Control Training Exercises compared to other general exercises in chronic nonspecific neck pain were found to be more effective (Carmen Martin Gomez et al.) [10]. Recent study have shown benefits of application of resistance exercise, mindfulness based and motor control exercises in reducing chronic neck pain [11]. However studies have also shown in recent times that there were insignificant outcomes of using IASTM and manual therapy in patients with chronic neck pain among college going students( Fatima Shewail et al.) [12].

## CONCLUSION

The pilot study to check the efficacy of the KIASTM along with Motor Control Training Exercises in chronic nonspecific neck pain has shown significant improvement in reducing pain, enhancing neck mobility, deep neck muscle endurance, and disability level. The participants showed positive outcomes after a single session of the protocol. However future studies with large sample sizes and considering the psychological status assessment of the patients might give a wider perspective of chronic nonspecific neck pain. Also future studies could be conducted by keeping a control group to check the efficacy of the treatment protocol.

**CONFLICT OF INTEREST**
No conflict of interest.

**SOURCE OF FUNDING**
Self

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**Table 1: Griffith MCE protocol**

***Motor Control Exercises***

Posture correction techniques taught in context of functional and work activities:

a) Active range of motion exercises

b) Isometric cranio-cervical flexion exercises performed sitting standing or lying.

c)An isometric cranio-cervical flexion exercise in an inclined sitting position with a head lift off of the supporting surface.

d)Isometric holds for up to 10 seconds repeated 10 times.

e) Participants also taught to engage deep neck flexors during functional activities.

f) Progression at the discretion of the treating therapist.

Patients were also asked to repeat the exercises 5 to 10 times per day.

**Figure 2: Results of Paired Sample T Test**

| Scores | Mean | SD | Correlation | t-test | p-value |
|---|---|---|---|---|---|
| Pre Intervention VAS | 7.40 | 0.55 | 0.67 | 11.18 | <0.001 |
| Post Intervention VAS | 2.40 | 0.55 | | | |
| Pre Intervention DNS | 12.62 | 4.47 | 0.86 | -7.29 | <0.001 |
| Post Intervention DNS | 20.20 | 3.64 | | | |
| Pre Intervention NDI | 39.94 | 5.12 | 0.61 | 12.01 | <0.001 |
| Post Intervention NDI | 14.70 | 0.76 | | | |

| | | | | | |
|---|---|---|---|---|---|
| Pre Intervention CF | 47.20 | 5.12 | -0.27 | -3.86 | 0.02 |
| Post Intervention CF | 57.60 | 2.07 | | | |
| Pre Intervention CE | 59.60 | 9.40 | 0.75 | -3.47 | 0.03 |
| Post Intervention CE | 69.20 | 6.65 | | | |
| Pre Intervention RLF | 44.00 | 2.65 | 0.12 | -3.57 | 0.02 |
| Post Intervention RLF | 48.60 | 1.52 | | | |
| Pre Intervention LLF | 36.60 | 4.78 | -0.25 | -2.21 | 0.09 |
| Post Intervention LLF | 44.40 | 5.18 | | | |
| Pre Intervention RLR | 54.00 | 7.52 | 0.80 | -2.09 | 0.10 |
| Post Intervention RLR | 59.40 | 9.71 | | | |
| Pre Intervention LLR | 54.20 | 2.59 | -0.38 | -2.41 | 0.07 |
| Post Intervention LLR | 63.00 | 6.82 | | | |

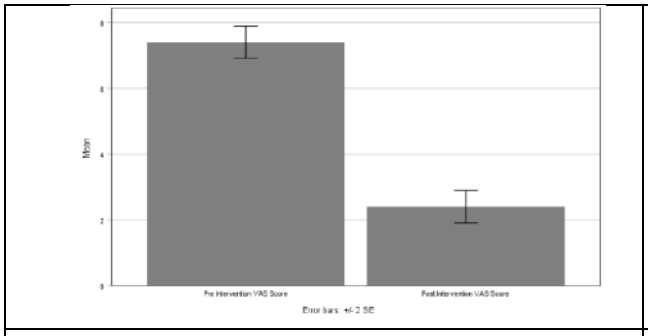


**Figure 1: Mean score for VAS**

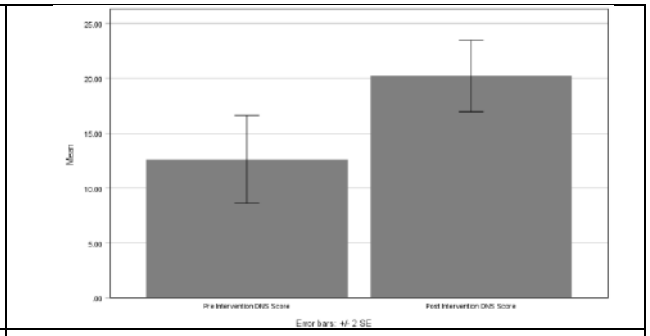


**Figure 2: Mean score for DNS Score**

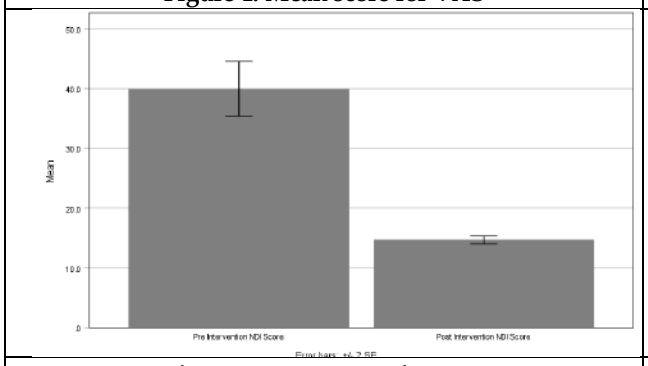


**Figure 3: Mean score for NDI**

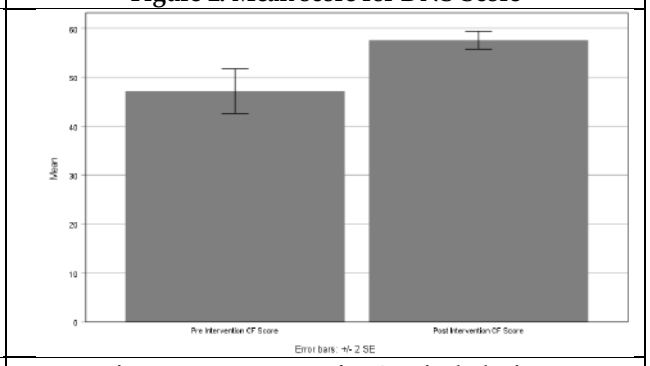


**Figure 4: Mean score for Cervical Flexion**

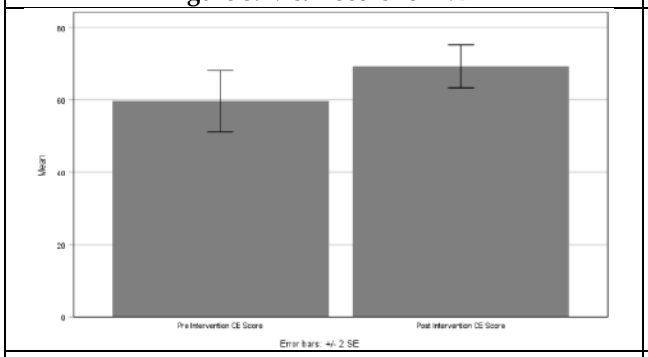


**Figure 5: Mean score for Cervical Extension**

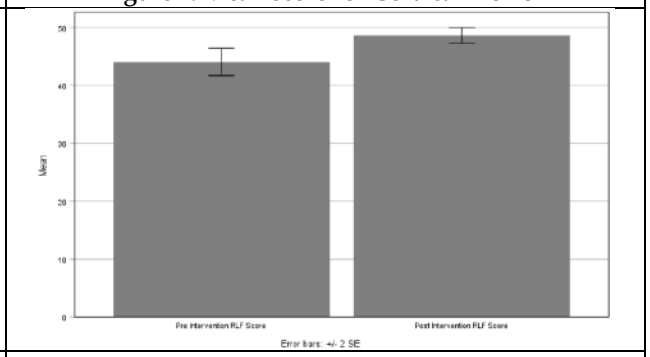


**Figure 6: Mean score for Right Lateral Flexion**

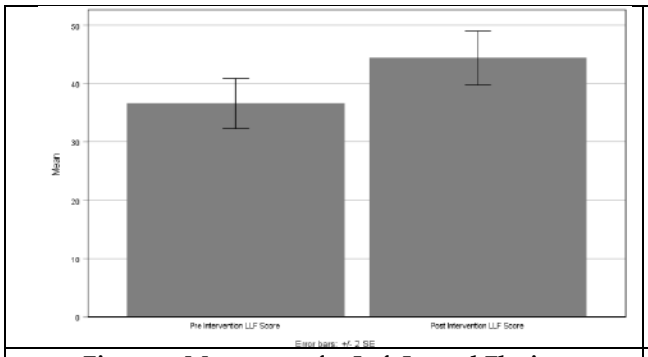


**Figure 7: Mean score for Left Lateral Flexion**

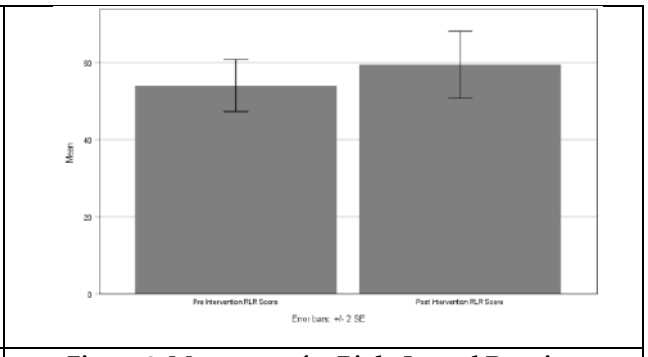


**Figure 8: Mean score for Right Lateral Rotation**

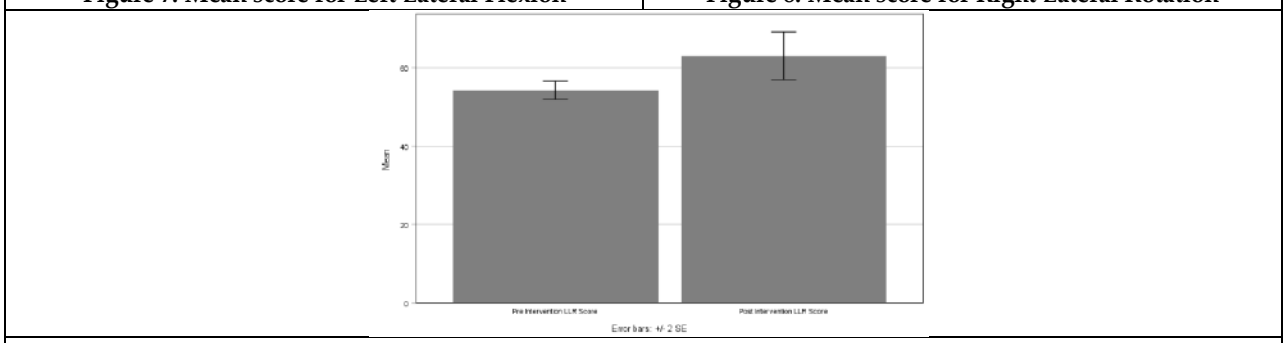


**Figure 9: Mean score for Left Lateral Rotation**

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**RESEARCH ARTICLE**

# Beyond the Plate from 3D to 4D: Revolutionizing Food Fabrication with Advanced Printing Technology

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## ABSTRACT

3D and 4D food printing technology represents a transformative advancement at the intersection of culinary arts, engineering, and materials science. The food industry is witnessing constant evolution in trends and innovations, mirrored by advancements in technologies like 3D food printing which involves transforming a digital 3D model into tangible edible objects using food materials. An extensive literature survey was conducted using databases PubMed, Science Direct, Google Scholar. Articles were searched using terms related to "3D and 4D Food Fabrication Technology". Total 53 articles from 2015 to 2023 were reviewed in order to write this review paper. This review article explores the principles, applications, and implications of these innovative technologies. To date, over 30 food items have been effectively fabricated through printing techniques. These include confectioneries like chocolates and fudge, baked goods such as biscuits and cakes, snacks like potato chips, fruit and vegetable items including diverse purees and juices, as well as jellies, meat products, and dairy products like cheeses and yogurt. Building upon the foundation of 3D printing, 4D food printing introduces an additional dimension of dynamic functionality. Through the integration of smart materials and responsive design principles, 4D printed food items exhibit the ability to transform or adapt their shape, texture, or taste in response to external stimuli such as temperature, moisture, or pH levels. This capability opens new avenues for culinary creativity, culinary experiences, and functional foods with enhanced sensory properties or therapeutic benefits. Beyond its culinary applications, 3D and 4D food printing hold promise for medical, nutritional, and environmental applications. Researchers are exploring the potential of 3D printed foods for personalized nutrition interventions, therapeutic diets, and dysphagia management. Food printing has the capability to adjust the nutritional composition of food to meet consumer preferences accomplished by incorporating healthy ingredients such as cellulose, plant chemicals, and high-quality proteins, while minimizing

adverse substances like anti-nutritional factors and allergens. The nutritional content of printed food can be managed strategically by utilizing fruits and vegetables as part of the food ink to provide essential micronutrients, cellulose, and other nutrients. However, several challenges include optimizing printing processes for scalability efficiency, safety, regulatory compliance, concerns related to taste, texture, and consumer acceptance and ethical considerations and the potential impact on traditional food systems warrant careful examination. 3D and 4D food printing technology represent a paradigm shift in food production and consumption, offering unprecedented opportunities for customization, sustainability, and innovation. As these technologies continue to mature, interdisciplinary research collaboration to unlock their full potential in the food and health sector is the way forward.

**Keywords** – 3D Food Fabrication, 4D Food Fabrication, Food Technology.

## INTRODUCTION

The food industry is witnessing constant evolution in trends and innovations, mirrored by advancements in technologies like 3D food printing. This technology involves transforming a digital 3D model into tangible edible objects using food materials. The feasibility of printing food depends on factors like the type of printer, the characteristics of printable inks, post-processing methods, and more.3D printing, also known as additive manufacturing (AM) and rapid prototyping (RP), is a burgeoning digital technology that sparks daily discussions, captivating researchers, industries, and the public alike due to its expanding range of applications in fields such as medicine, gastronomy, engineering, manufacturing, art, and education. Among these, gastronomy stands out as a significant area of interest and challenge. The current focal point of interest in 3D printing is "3D food printing." This process involves controlled robotic manipulation, where products are constructed layer by layer using computer-aided design (CAD) programs or downloaded models from online platforms.

Once a 3D model is created, the design data is transmitted to the printer, which then divides the model into layers and constructs them according to the specified cross-sectional pattern. In the realm of food, there's a belief that RP technology will redefine food processing by tailoring products to meet specific consumer preferences regarding taste, cost, convenience, and nutrition. Additionally, it's seen as a tool to democratize innovation by lowering barriers to entry for inventors, fostering a new class of independent designers and a custom product economy. Despite the complexity of food systems and their varied properties, researchers have been exploring the application of 3D printing to different types of food products. While the engineering principles behind 3D food printers are understood, the creative potential of this technology remains largely untapped. Through optimization of various printing parameters and ingredients, researchers have achieved successful outcomes in printing with a range of food substrates, including chocolate, cookie dough, cereals, sugar powder, processed cheese, meat gels, and even fruits and vegetables.

However, despite numerous studies and reviews on 3D food printing, gaps still exist in understanding the relationship between printing process variables and material structure to achieve desired outcomes. 4D printing technology, an evolution of 3D printing, represents a novel concept where the printed configuration undergoes changes over time (Choi et al., 2015).[1] This concept was initially pioneered by a research team at the Massachusetts Institute of Technology (MIT) in 2013 (Tibbits, 2014). [2] In terms of the printing process, 4D printing closely resembles 3D printing, involving stages such as 3D design development and fabricating the structure using a 3D printer (Choi et al., 2015). [1] However, the key distinctions between 4D and 3D printing lie in smart design and smart materials, as 4D printed structures have the capability to alter their shape or function (Pei & Loh, 2018). [3]This review aims to deliver deeper into optimizing extrusion-based food printing, focusing on 3D food printing process, its advantages and also addressing its limitations and offering insights to overcome barriers in this fieldand extension to 4D Food Printing Technology and its future aspects.

## METHODOLOGY

An extensive literature survey was conducted using databases PubMed, ScienceDirect, Google Scholar. Articleswere searched using terms related to "3D and 4D Food Fabrication Technology". Total 53articles from 2015 to 2023 were reviewedin order to write this review paper. This study was approved by Publication Guidelines and Monitoring Committee (PGMC) Approval from Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India for Publication.

## DISCUSSION

**FEEDING THE FUTURE: EXPLORING 3D FOOD FABRICATION TECHNOLOGY**

The fantastical 3D printing depicted in sci-fi classics like Star Trek, Jurassic Park III, and Westworld has transitioned into real-world applications. Now, we witness the fabrication of 3D printed cars, skulls, hearts, prosthetics, and more. A milestone in this progression occurred in 2006 when Cornell University developed the first multi-material 3D printer capable of printing edible items like chocolates, cookie doughs, and cheeses (Liu et al., 2017). [4] Since then, the utilization of 3D food printing has surged, not only in research settings but also in commercial domains, driven by cost reductions and technological advancements.

3D printing, also referred to as additive manufacturing, involves creating a three-dimensional object using either a computer-aided-design (CAD) model or a digital 3D model. This process entails adding raw materials, depositing them, and solidifying them, typically in layers under the control of a computer. In the realm of 3D food printing, the raw materials are food ingredients, and a typical printer comprises three main components: a printing system, an operational control platform, and a food capsule. The food capsule contains the food material, often a thick slurry similar to printer ink, which is pushed through a food-grade nozzle by a syringe pump to be deposited layer-by-layer onto a platform according to a pre-designed shape controlled by a computer. Whether the printed food can be consumed directly or requires further cooking depends on the nature of the food ingredients and the sanitary condition of the printing device.

**PRINTED PALATES: UNVEILING THE 3D FOOD PRINTING PROCESS**

3D food printing, also known as food fabrication or additive manufacturing in the culinary world, involves the use of specialized 3D printers to create edible items layer by layer. Here's a general overview of the process:

**Flow Chart Representing 3D Food Printing Process**

- **Design**: The process begins with the creation of a digital model or design of the food item to be printed. This can be done using computer-aided design (CAD) software or by using specialized food design software.
- **Preparation of Ingredients**: Edible ingredients are prepared in a form suitable for extrusion through the printer's nozzle. These ingredients can include various food materials such as dough, batter, purees, gels, or pastes. Depending on the printer and the desired food item, these ingredients may need to meet certain consistency and texture requirements.
- **Loading Ingredients**: The prepared ingredients are loaded into the printer's cartridges or syringes. Some 3D food printers may have multiple cartridges to enable printing with different ingredients or colors.
- **Printing**: The printer follows the digital model or design and deposits the edible material layer by layer onto a build platform. This is typically done through a process called extrusion, where the material is pushed through a nozzle or syringe. The printer's movements are controlled precisely to ensure accurate deposition of each layer.
- **Layer-by-Layer Building**: The printer continues to build up the food item by adding successive layers of edible material. The layers may be very thin, depending on the printer's resolution and the complexity of the design.
- **Post-Processing (Optional)**: After printing is complete, some food items may require additional processing steps such as baking, cooling, or assembly before they are ready to be served.

- **Serve and Enjoy**: Once any necessary post-processing steps are complete, the 3D printed food item is ready to be served and enjoyed.

It's important to note that 3D food printing technology is still relatively new and continues to evolve. While it has the potential to revolutionize aspects of food production, it is currently mainly used in niche applications such as custom confectionery, personalized nutrition, and food customization for people with specific dietary needs or restrictions. Additionally, the taste, texture, and overall quality of 3D printed food items can vary depending on the ingredients used, the printer's capabilities, and the skill of the chef or operator.

**EDIBLE BLUEPRINTS: ASSESSING THE SUITABILITY OF FOODS FOR 3D PRINTING**

To date, over 30 food items have been effectively fabricated through printing techniques. These include confectioneries like chocolates and fudge, baked goods such as biscuits and cakes, snacks like potato chips, fruit and vegetable items including diverse purees and juices, as well as jellies, meat products, and dairy products like cheeses and yogurt (Dankar et al., 2018). [5]

From a technological standpoint, the flow and deformation characteristics, referred to as rheological properties, of the "food ink" are paramount in 3D food printing. For successful extrusion from the nozzle, the ink must be sufficiently fluid, while also providing structural support upon deposition to uphold the shape of the printed item (Chen et al., 2022).[6] In rheology, inks exhibiting such traits are termed Bingham plastic or Herschel-Bulkley materials. These materials form a delicate network at rest, supporting their weight to maintain shape. When subjected to a significant stress, known as yield stress in rheology, the network breaks down, allowing the ink to flow for printing (Chen et al., 2022).[6]When analyzed using a rheometer, most printable materials demonstrate shear thinning behavior, meaning they have lower viscosity at higher deformation rates and higher viscosity at lower deformation rates (Chen et al., 2022).[6]In addition to formulation, temperature serves as a practical means to regulate ink rheology (Chen et al., 2022).[6] For instance, heating chocolates above their melting point facilitates flow and subsequent printing. Subsequent solidification upon storage at ambient or lower temperatures enables network formation (high viscosity or yield stress) and maintains the desired shape.Moreover, texture significantly influences the sensory and structural attributes of printed products, with reported hardness values ranging from 4.48 to 59.8 Newtons and springiness values typically below 1 for successful prints (Zhang et al., 2021).[6] Physical and chemical transformations of constituent ingredients during storage and thermal processes are also expected to impact the sensory and quality attributes of printed items.

**EXPLORING THE PROS AND CONS OF CONTEMPORARY 3D FOOD PRINTING TECHNOLOGY(Pereira et al., 2018)[7]**

Benefits of contemporary 3D food printing technology include:Tailored nutrition and health benefits for individuals, Customized creation of intricate visual designs and textures, Personalized ingredient selection to prevent allergies and cross-contamination, Reduction of food wastage by conserving materials, Opportunity to utilize alternative material sources, Streamlining and expediting the manufacturing process, Conservation of energy and reduction of transportation requirements. Meanwhile, drawbacks of present-day 3D food printing technology encompass: Expenses related to the printer and "food ink", Insufficiency of suitable "food inks" for 3D printing, Sluggish printing speed and challenges for large-scale production, Safety apprehensions regarding printers and "food inks", Considerations regarding consumer perception, Challenges concerning printing precision and surface finishes.[8]

**BEYOND BOUNDARIES: EVOLVING TO 4D FOOD FABRICATION TECHNOLOGY**

Four-dimensional (4D) printing represents a recent advancement in additive manufacturing techniques, extending from 3D printing by introducing the concept of altering printed configurations over time (Choi et al., 2015).[1] Initially conceptualized by a team at the Massachusetts Institute of Technology (MIT) in 2013 (Tibbits, 2014)[2], 4D printing shares similarities with 3D printing in terms of design development and structure printing processes using a 3D printer (Choi et al., 2015).[1] However, the distinguishing features of 4D printing lie in the integration of smart design principles and smart materials, enabling printed structures to change shape or function (Pei & Loh, 2018).[3]

Smart materials employed in 4D printing exhibit properties such as self-assembly, versatility, and self-healing capabilities (He, Zhang, & Guo, 2020).[9] Most commonly used 4D printing materials are single or multilateral polymers, including shape memory polymers, liquid crystal elastomers, and composite hydrogels (Sharma &Tabbits, 2020).[10] The responsive shape changes of 4D printing structures offer advantages in terms of space-saving during transportation and storage (Pei & Loh, 2018),[3] leading to exploration across various fields such as robotics, biomedical applications, tissue engineering, and electronic devices (Ghazal et al., 2021).[11] However, the application of 4D printing in the food sector remains relatively limited, with only a few studies available, indicating an early stage of development (Chu et al., 2020).[12].

The adaptability of materials in 4D printing allows for customized responsiveness configurations, contributing to material reduction through the self-changing nature of 4D printed objects (Chu et al., 2020).[12] Additionally, 4D printing has the potential to address consumer demands for unique food products (Phuhongsung, Zhang, & Bhandari, 2020).[13] Furthermore, the directed and adjustable deformation of structures in 4D printing could assist chefs in determining optimal cooking points, enhancing the visual appeal and taste of food items (Chen et al., 2021).[14] It's worth noting that while 4D printing allows for achieving desirable product properties within an appropriate timeframe, these properties may diminish during the storage of printed products.

**TIME TO TASTE: UNVEILING THE POTENTIAL OF 4D PRINTING IN FOOD**

In the realm of food technology, researchers at MIT pioneered the use of 4D printing. They devised a method involving a 2D film made from a blend of starch, cellulose, and protein, activated by water. This 2D film transformed into a 3D structure upon exposure to water (Wang et al., 2017).[15] The application of 4D printing in food holds promise for tailoring products and cultivating distinct flavor profiles. By manipulating the composition of printing ink with different food materials and formulas, the characteristics of the resulting 4D printed food can be customized (Teng et al., 2021).[16] Variations in temperature and pH can induce alterations in the color, texture, aroma, and shape of food. For instance, when microwave heating was employed, a 4D transformation in 3D printed buckwheat dough and lotus root powder gel was observed (Guo et al., 2021; Chen, Zhang, Mujumdar, et al., 2021).[17,18] Similarly, shifts in pH levels were found to affect the color of a mixture composed of 3D printed soy protein isolate, pumpkin, and beetroot (Phuhongsung, Zhang, &Devahastin, 2020a).[19].

**INNOVATIVE INKS: EXPLORING THE ROLE OF INKS IN 4D FOOD PRINTING**

In recent times, printing technology has been instrumental in the development of a wide variety of food products. These include chocolate (Rando &Ramaioli, 2021)[20], soybean (Phuhongsung, Zhang, &Devahastin, 2020b; Balla et al., 2020)[21,22], meat (Wilson et al., 2021; Wilson et al., 2021), starch (Zheng et al., 2021; Zeng et al., 2021), fruits, vegetables (Chen et al., 2021; Chen et al., 2021), and food hydrocolloids (Pant et al., 2021), among others.[23-29] The moisture content of these food materials, crucial for their printing performance, is significant. Therefore, utilizing dried and powdered forms of food materials can preserve their nutritional value and functional properties (Lee et al., 2019).[30]Furthermore, the food ink mixture utilized in 4D printing comprises specific materials designed to respond to stimuli. These materials undergo changes in color, flavor, texture, and more in response to certain conditions or stimuli. Therefore, the sensitivity of these materials in food printing ink is crucial for achieving 4D transformations in printed objects. The pigments within the printing ink are responsible for altering the color of food in response to varying pH levels. For instance, curcumin, a stimulus-responsive material employed in 4D printing, displays a red hue in alkaline pH environments and a yellow hue in acidic or neutral pH environments (C. Chen, Zhang, Guo, et al., 2021).[31].

Likewise, anthocyanin functions as a responsive substance, displaying diverse hues in response to pH alterations (He et al., 2021). Similarly, the gelatin-gum Arabic-flavor oil complex has demonstrated thermal responsiveness, serving as a stimulus-reactive material in the printing of buckwheat dough with yellow flesh peach. In their study, Guo et al. (2021) noted concurrent alterations in both color and flavor of the product when subjected to microwave stimuli. It is imperative that the changes induced by stimuli in 4D printed objects remain manageable, as unregulated changes could lead to undesirable product outcomes. To ensure controlled alterations in the properties of 4D printed objects

under varying stimuli, strategic design coding and meticulous distribution, such as uniform, gradient, or patterned dispersion of components, are essential.[32,33]

**FROM CONCEPT TO CUISINE: NAVIGATING MODEL DESIGN AND PRINTING PROCESSES IN 4D FOOD PRINTING**

4D printing involves the transformation of a 3D printed model through external stimuli. Therefore, the design of the model holds paramount importance in the 4D printing procedure. Effective software plays a crucial role in facilitating 4D printing by allowing the production of the intended product. The 4D model design and printing process entail the utilization of various software solutions, including separate solutions for Simulation, Modeling, Slicer, Host/Firmware, Monitoring, and one Printing Management software, as depicted in.

Simulation involves replicating the behavior of an object or system using computer software. It is commonly utilized in the preliminary stages of product development to mitigate potential failures before physical production. By employing mathematical formulas to mimic real-world phenomena, simulation aids in comprehending the entire operation without practical execution. In the realm of 4D printing, simulation techniques can be categorized into two: finite element analysis (FEA) software and software dedicated to simulation function development. Mao et al. (2015) exemplified this by employing FEA simulations via ABAQUS software, incorporating a user-defined constitutive model, and utilizing a reduced-order model (ROM) to simulate rigid body motions and explore folding pathways. After the simulation process, the 3D design of the printing object is crafted using modeling software. Modeling entails creating a representation of the object with its pertinent characteristics, often resembling the actual item. This process results in a 3D rendition of the object. Commonly employed computer-based graphics and Computer-aided design (CAD) software for this purpose in food printing include Rhino 6 software, Rhinoceros 5.0 (Liu et al., 2021), and SketchUp Pro 2015 (Ghazal et al., 2021), among others. Notably, the modeling software does not directly transmit information regarding the 3D design to the printer. Instead, slicer software is utilized to convert the model into 2D sections containing the necessary information for the printer.

Slicer software is a computer application widely utilized in the realm of 3D printing to transform a 3D object model into precise instructions tailored for the printer. It incorporates various editing tools such as rotation, scaling, as well as import and export functionalities for CAD files. Furthermore, it features a subdivision function compatible with Stereo Lithography (STL) files. This allows for the conversion of a low-resolution image to a higher resolution one by enhancing intricacy and smoothness (Chung et al., 2017). The slicer software generates the blueprint for printing, known as G code, which encapsulates all the essential information defining the entire printing process. Common slicing software utilized in food printing includes Slic3r, Simplify3D, and others. After the slicer software prepares the G-code (slicer data), it proceeds to transmit it to the printer. Subsequently, the host/Firmware software, when connected to a Personal Computer (PC), generates the 3D object. Essentially, the host software assumes control over the printer's components, including the printer's head and bed, analyzes the received G-code data, and dispatches G-code instructions to the printer.

In the context of 4D printing, the synthesized 3D object undergoes gradual changes in its properties over time, typically triggered by specific environmental conditions. Monitoring software facilitates the observation of these stimulus-induced self-transformations in 4D printed objects and initiates necessary actions if required. This monitoring software can be installed on user devices and monitored continuously. The choice of material significantly influences the design and functionality of 4D printed objects. The material must exhibit responsiveness to specific stimuli throughout the layer-by-layer printing process. The selection of material dictates the stimuli necessary to trigger the self-transformative changes in the printed objects. Upon exposure to stimuli, only specific components of the printed object interact, leading to predetermined transformations. Interaction mechanisms between the 4D printed object and the stimulating agent may involve mechanical loading or physical manipulation, resulting in a sequence of changes. Mathematical modeling determines the required exposure to stimuli to achieve desired changes in the printed object's properties (Ahmed et al., 2021). Achieving a shape-shifting effect with a single material involves manipulating the gradient distribution of the material by strategically controlling the spatial

position and morphology of the lattice structure throughout the product (Pei & Loh, 2018). The self-induced changes in the material's shape depend on its responsiveness to stimuli and the physical design geometry of the material (Lendlein& Kelch, 2002). The 4D printing process comprises distinct steps, each involving different software. The output of one step may serve as input for subsequent steps, thereby influencing the next stage. Consequently, the software utilized in each step plays a specific role and may impact subsequent stages. All stages are interconnected, forming a network, and thus, all software in each processing stage is monitored and controlled by the user. This is facilitated through a printing management system connected to each software at every stage, designed to optimize printer usage (Chung et al., 2017).[34-42]

Numerous intriguing instances demonstrate that through the manipulation of food chemistry and physics, 4D-printed foods can offer innovative concepts for crafting interactive culinary experiences. One such example involves the curvature of purple sweet potato purees during microwave dehydration, with the ability to adjust the rate by altering microwave power, salt content, or fructose syrup content, thereby yielding personalized shapes (He et al., 2020).[8]

**INKJET PRINTING**

Inkjet printing, a common technique in 4D printing, is frequently employed in confectionery and decorative applications (Pallottino et al., 2016). This method involves a series of pneumatic membrane nozzles, typically ranging from 20 to 50 μm, which dispense small droplets of printing material onto a moving substrate. Both single and multiple nozzles can operate simultaneously, allowing printing ink to be sprayed onto the printing platform, creating a layered structure. These droplets amalgamate to form a digital image with surface fillings and cavity depositions. Inkjet printing typically utilizes low-viscosity materials, making it more suitable for producing flat products rather than intricate structures. Temperature plays a significant role in inkjet printing as it affects the viscosity and surface energy of the material (Le-bail et al., 2020).

Robert John Young introduced a machine incorporating a bubble-jet printer head and a reservoir for liquid food colorant, which is utilized to print images onto the surface of an edible substrate, as previously outlined. This innovation also included a method for printing images onto edible substrates (Robert John Young, 2000). Similarly, Pallottino et al. developed a device to improve the printing of low-viscosity substances on an edible surface by modifying the surface with a high-polarity water-based glaze or by polishing the gum surface (Pallottino et al., 2016). Inkjet printing is primarily suited for low-viscosity substances, limiting its applicability in producing complex food structures. Its uses extend to graphical decoration, fillings, micro-encapsulation, and to some extent, 3D nano printing (Fernanda C. Godoi et al., 2018).[43-47]

**NUTRITION BY DESIGN: UNDERSTANDING NUTRITIONAL CHANGES IN THE PRINTING PROCESS**

Food printing has the capability to adjust the nutritional composition of food to meet consumer preferences. This can be accomplished by incorporating healthy ingredients such as cellulose, plant chemicals, and high-quality proteins, while minimizing adverse substances like anti-nutritional factors and allergens. The nutritional content of printed food can be managed by strategically distributing nutrients in the printing ink or alternatives during the printing process. Utilizing fruits and vegetables as part of the food ink can provide essential micronutrients, cellulose, and other nutrients (Chen et al., 2022). Incorporating tissue engineering into food printing holds promise for enhancing the nutritional aspects of 4D printing. Substituting ink and tissue with food-grade materials in 4D biotechnological printing can elevate the nutritional quality of printed food, as exposure to appropriate conditions stimulates tissue growth. The printed object may contain plant or animal cells capable of forming tissue-like structures under suitable stimuli and producing nutrients (Teng et al., 2021). Additionally, printing food with probiotics can enhance the nutritional quality of the printed object. The inclusion of microalgae offers supplementary nutritional and functional components such as protein, fatty acids, sterols, and vitamins (Uribe-Wandurraga et al., 2020).

UV irradiation of 4D printed purple sweet potato pastes infused with ergosterol resulted in a notable increase in vitamin D2 content, particularly in the irradiated area (Chen et al., 2021). Similarly, microwave stimulation of

beeswax-based oleo gel incorporated with purple potato powder ink produced a low-fat printed product (Shi et al., 2022).[48-53]

## CONCLUSION

4D food printing is currently in its early stages, presenting ample opportunities for extensive research. Given its infancy, traditional 3D printing equipment is commonly utilized, but there is a need for the development of new printing conditions or equipment with enhanced functionality to better simulate 4D printing. This advancement would facilitate the achievement of desired changes, such as deformation and denaturation of various components of food ink. Currently, researchers primarily focus on microwave heating and changes in pH as stimuli sources. However, there is potential for exploration of new stimulus agents like light and changes in ionic concentration in 4D food printing. Moreover, the range of stimulus-response materials studied in 4D food printing is limited. Investigating new components such as diacetyl and vanillin could broaden the scope. Additionally, employing multiple stimulus-responsive materials to induce simultaneous changes in multiple food properties would represent a significant breakthrough in 4D food printing. The development of a monitoring system to assess the degree of 4D changes could play a crucial role in achieving controlled transformations in 4D food printing.

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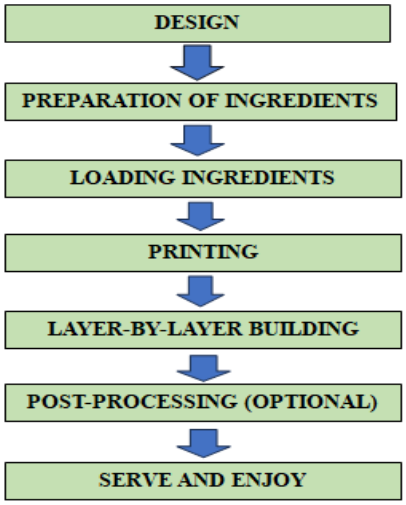


**Flow Chart Representing 3D Food Printing Process**

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**RESEARCH ARTICLE**

# Challenges and Opportunities in Implementing Sustainable WASH Practices in Government Schools: Lessons from Tamil Nadu

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## ABSTRACT

The paper explores the challenges and opportunities associated with implementing sustainable Water, Sanitation, and Hygiene (WASH) practices in government schools in Tamil Nadu, India. Despite significant advancements in policy frameworks and financial investments aimed at improving WASH infrastructure, many schools still struggle with inadequate facilities, poor maintenance, and limited community engagement. The study identifies critical barriers such as financial constraints, socio-cultural factors, and gaps in policy execution. The research highlights innovative approaches and best practices that have successfully addressed these challenges in certain schools. Key opportunities include leveraging community participation, integrating WASH education into the curriculum. The findings underscore the importance of a holistic approach that combines government support, community involvement, and sustainable practices to enhance the overall effectiveness of WASH initiatives in schools in Tamil Nadu.

**Keywords**: WASH, Challenges, Menstrual Hygiene, Sustainable development, education, Community Participation.

# INTRODUCTION

WASH is an abbreviation for "Water, Sanitation and Hygiene". It encompasses the provision of clean water, sufficient sanitation facilities, and the promotion of proper hygiene practices to enhance public health and well-being. Water, sanitation, and hygiene (WASH) involves guaranteeing the availability of clean and affordable water for drinking, cooking, and personal and domestic hygiene. It also includes providing access to facilities and services for the safe disposal of human waste, as well as promoting behaviors and practices that contribute to cleanliness and good health, such as hand washing with soap, managing menstrual hygiene, and practicing food hygiene.

Tamil Nadu, a state renowned for its commendable literacy rate, encounters substantial obstacles in effectively implementing sustainable Water, Sanitation, and Hygiene (WASH) practices in its government schools. Although the state has a high literacy rate, with 87% for males and 73% for females according to the 2011 census, it still faces challenges regarding sanitation and hygiene, especially in rural regions. A significant cause for girls dropping out of schools is the lack of awareness and understanding regarding menstruation and menstrual customs. It is estimated that over 23 million girls leave school each year as a result of this issue. Furthermore, a staggering 79% of females in Tamil Nadu lack knowledge on menstrual cleanliness and habits, rendering them vulnerable to diseases. The state has made substantial progress in developing infrastructure, having erected over 48 lakh toilets in rural areas since 2014, resulting in the state being declared open-defecation free. Nevertheless, the upkeep and utilization of these facilities continue to be a cause for concern. A total of 7837 government schools are without operational toilets, and even in cases where toilets are present, they are frequently inadequately kept. The implementation of initiatives such as the Swachh Bharat Swachh Vidyalay scheme aims to enhance WASH standards. However, their effectiveness is constrained by insufficient awareness and a lack of behavioral modification.

The state's Vision 2023 Project seeks to promote collaborations between the public and commercial sectors to develop infrastructure, which may be utilized to raise awareness through digital interventions. Tamil Nadu's rural areas have achieved a significant internet penetration rate of 41.98%, which creates a favorable environment for utilizing Information and Communication Technology (ICT) to raise awareness and encourage changes in behavior. Efficient policies and digital interventions can have a vital impact in tackling these difficulties and guaranteeing the continuity of WASH practices in public schools, eventually promoting high-quality education and gender parity.

Attaining high-quality education at the school level is an ongoing and adaptable process that requires periodic adjustments to align with the specific requirements of the social environment. An example of such an aspect is a policy that guarantees a comprehensive education system aimed at fostering gender equality starting from the very basic level. An intervention is required to improve the infrastructure for maintaining sanitation and hygiene for female students. Additionally, there is a need for a comprehensive understanding of gender issues through awareness, which can result in meaningful and natural social change. The state has a clear responsibility to guarantee high-quality education and address any deficiencies in the education system through the implementation of innovative approaches. Tamil Nadu exhibits exceptional performance in terms of literacy rate. Nevertheless, there are still tangible societal obstacles that require intervention and tailored approaches. Tamil Nadu ranked third in the 2011 census, following Kerala and Maharashtra. The male literacy rate stood at almost 87%, while the female literacy rate was approximately 73%. Sanitation and hygiene are the primary aspects that are crucial in guaranteeing the "quality" of education for girls. This element specifically includes three primary Sustainable Development Goals (SDGs) of the United Nations: Quality Education, Gender Equality, and Water & Sanitation. Ensuring access to clean water, sanitation, and hygiene (WASH) in rural communities is a crucial step towards achieving gender equality and excellent education.

**Objectives**

The study seeks to identify the primary obstacles that hinder the adoption of sustainable WASH practices in government schools in Tamil Nadu. Additionally, it aims to evaluate the impact of socio-cultural factors on the

implementation and sustainability of WASH facilities at these educational institutions. In addition, the project will assess the efficacy of existing policy frameworks and financial initiatives in enhancing WASH infrastructure. The program will examine novel methodologies and exemplary strategies that have effectively tackled WASH-related difficulties in certain government schools. Moreover, the study will investigate the impact of community involvement on improving the long-term viability of WASH practices. Additionally, it will examine the incorporation of WASH education into the academic program of schools and its influence on the behavior of both students and staff, with the aim of enhancing WASH facilities.

**Statement of problem**

Despite substantial investments and regulatory frameworks aimed at improving Water, Sanitation, and Hygiene (WASH) infrastructure in government schools, Tamil Nadu continues to face significant challenges in implementing sustainable WASH practices. The extensive implementation of WASH initiatives is impeded by obstacles such as poor infrastructure, insufficient upkeep, and limited involvement of the community. The issues are exacerbated by financial constraints, socio-cultural factors, and deficiencies in policy implementation. The study seeks to examine the specific challenges and opportunities related to the adoption of sustainable WASH practices in government schools in Tamil Nadu. The study aims to comprehensively comprehend the successful introduction, maintenance, and scaling of sustainable WASH practices in the educational sector in Tamil Nadu. This will be achieved through analyzing the challenges and identifying effective approaches and best practices.

**Significance of the study**

The study's importance rests in its capacity to influence the health, education, and overall welfare of kids at government schools in Tamil Nadu. The research seeks to enhance the learning environment and reduce absenteeism by improving the quality of WASH facilities through identifying and addressing the issues associated with implementing sustainable WASH practices. Moreover, the study's understanding of socio-cultural elements and policy deficiencies can contribute to the development of more efficient and situation-specific remedies. In essence, this study contributes to the overarching objective of attaining Sustainable Development Goal 6, which prioritizes universal access to clean water and sanitation. It achieves this by promoting sustainable WASH practices that can be upheld in the long run specifically in government schools in Tamil Nadu.

**Theoretical framework**

The study on the challenges and opportunities in implementing sustainable WASH practices in government schools in Tamil Nadu is based on a theoretical framework that incorporates various key theories and models. This framework aims to provide a thorough understanding of the factors that influence WASH practices and the methods by which sustainable implementation can be accomplished. Behavior Change Theory, such as the Health Belief Model and the Theory of Planned Behavior, aids in comprehending the behaviors of individuals and communities in relation to WASH practices. It does so by examining how attitudes, beliefs, and social norms impact the adoption and continuation of hygiene practices. Systems Theory offers a structure for comprehending the intricate interaction among different elements in WASH practices, highlighting the necessity of a comprehensive approach. The Socio-Ecological Model analyzes several levels of influence on WASH behaviors, ranging from individual to policy levels, emphasizing the significance of addressing these layers to establish a conducive environment.

The Sustainable Development Theory prioritizes the deployment of environmentally-friendly and cost-effective technology in WASH infrastructure to guarantee long-term sustainability. Public Policy Implementation Theory analyzes the process of turning policies into action, specifically focusing on identifying deficiencies in the execution of policies connected to WASH practices. The Community Participation Theory highlights the significance of community engagement in ensuring the effectiveness of WASH efforts. It suggests that when the community actively participates and takes ownership, it results in improved maintenance and long-term sustainability. This study seeks to combine different theoretical perspectives in order to gain a detailed understanding of the difficulties and possibilities involved in implementing sustainable WASH practices in government schools. It aims to identify the

main factors at different levels that affect WASH practices and provide valuable insights into effective strategies for overcoming obstacles and taking advantage of opportunities for sustainable implementation.

## METHODOLOGY

The study adopted a qualitative approach, utilizing both descriptive analysis and analytical methods to examine the challenges and opportunities in implementing sustainable WASH practices in government schools in Tamil Nadu. Interviews were conducted with teachers, principals, and NGO coordinators, supplemented by open-ended questions to elicit comprehensive insights. Thematic analysis was then applied to identify trends and patterns in the data collected from the interviews. The study incorporated secondary data sources including articles, books, state government reports, NGO reports, UNICEF official documents, and websites to enrich the analysis and provide a broader context for understanding the issues at hand. This comprehensive approach allowed for a thorough examination of the complexities surrounding WASH practices in Tamil Nadu's government schools, shedding light on key areas for improvement and potential avenues for intervention.

## RESULT AND DISCUSSION

**The Imperative of Menstrual Hygiene Awareness in Educational Institution**

The significance of being conscious about menstrual hygiene is of utmost importance, particularly within educational institutions and society norms. Addressing menstrual hygiene in schools is essential for ensuring girls' continued enrollment and decreasing dropout rates. As per a survey by the Dasra foundation, almost 23 million girls discontinue their education every year because they are not well-informed on menstrual hygiene. The high proportion of students leaving school without completing their education emphasizes the need for including menstrual hygiene education in school curricula. The dearth of consciousness among girls and women, as emphasized in Tamil Nadu where 79% were uninformed about menstrual hygiene habits, might result in severe health repercussions. Girls who lack adequate understanding are more vulnerable to infections during menstruation. The lack of awareness is frequently sustained by the stigma associated with menstruation, which is commonly regarded as unclean. Research has indicated that females frequently acquire knowledge about menstruation primarily from their mothers rather than from educational institutions. This suggests a deficiency in the process of incorporating the issue into the institutional framework of schools. By integrating menstruation education into the school curriculum, it is possible to normalize and eliminate the stigma associated with menstruation.

Utilizing Information and Communication Technology (ICT) in educational institutions can improve communication and facilitate the dissemination of knowledge regarding menstrual hygiene. This can be especially efficacious in rural locations where conventional procedures may not have the same level of effectiveness. Crucially, it is essential that menstrual hygiene instruction is not exclusively provided to girls. It is important to provide boys with education on menstruation in order to cultivate comprehension and empathy towards this matter. Schools can contribute to dismantling societal taboos and advancing gender equality by engaging boys in these talks. Understanding the importance of maintaining proper menstrual hygiene is crucial for promoting the well-being, educational opportunities, and empowerment of girls and women. Schools may play a crucial role in dismantling the social taboos associated with menstruation and fostering a more inclusive society by integrating menstrual hygiene instruction into their curricula and engaging both male and female students in the conversation.

**Challenges in Sustainable WASH Implementation in Tamil Nadu Schools**

Government schools in Tamil Nadu encounter numerous substantial obstacles in the implementation of sustainable Water, Sanitation, and Hygiene (WASH) practices. A significant issue is the insufficient awareness and understanding of menstruation and menstrual practices, which leads to girls dropping out of school. Approximately 23 million females discontinue their education annually as a result of this cause. Moreover, a staggering 79% of females in Tamil Nadu lack knowledge on menstrual cleanliness and habits, rendering them vulnerable to diseases.

Insufficient infrastructure for sanitation and hygiene poses a significant obstacle. Although Tamil Nadu has achieved the status of being free from open defecation, with the construction of more than 48 lakh toilets in rural areas since 2014, there are still a significant number of schools that do not have operational toilets. There are 7837 schools in the state that either has broken toilets or no toilets at all. This negatively affects learning and leads to students leaving school. The upkeep and utilization of current facilities also continue to be a noteworthy challenge. An additional major barrier is the absence of modifications in behavior and participation from the community in WASH activities. Although certain districts such as Thiruvallur and Vellore have displayed outstanding records in water, sanitation, and hygiene (WASH), the entire state must prioritize capacity building and behavioral change in order to achieve sustainable and enduring outcomes. This can be accomplished by conducting subsequent seminars that actively include individuals and groups, instructing them on the proper utilization of toilets and the successful maintenance of hygienic standards.

The incorporation of Information and Communication Technology (ICT) in WASH programs is crucial, yet there is sometimes a deficiency in comprehending its exact function. The Swachh Bharat Swachh Vidyalay program has facilitated the use of Information and Communication Technology (ICT), however its usefulness is limited due to the lack of clearly defined objectives and efficient implementation. In order to complement the state's focus on infrastructure development, it is necessary to undertake initiatives to increase knowledge and offer guidance on WASH practices. The Vision 2023 Project seeks to foster cooperation between the public and commercial sectors in order to enhance infrastructure development. This can be achieved by using digital interventions to increase awareness, taking advantage of the state's substantial rural internet penetration rate of 41.98%. Lack of coordination among departments and the degree of instructor motivation are vital elements that enable the sustained implementation of Water, Sanitation, and Hygiene (WASH) projects in schools. Ensuring effective collaboration between departments and maintaining elevated levels of teacher morale are crucial for the success of WASH initiatives. The government must prioritize Water, Sanitation, and Hygiene (WASH) to guarantee a good standard of education. Ensuring the availability of sufficient health and hygiene facilities and raising awareness among girls at the school level is essential for establishing a strong basis for delivering education of superior quality. Lack of knowledge and comprehension of menstruation and menstrual customs greatly contribute to the high dropout rate among females in schools. Implementing nationwide regulations for Water, Sanitation, and Hygiene (WASH) in schools is a significant challenge. The Ministry of Education should assume responsibility and set standards for adequate and comprehensive provision of clean drinking water, sanitation, and hygiene facilities in schools, while collaborating with other relevant ministries. The state must address the issue of disparity in the accessibility of clean water and sanitation. Effective WASH programs in schools that address this issue are essential for ensuring that every kid has equal access to these basic necessities.

The state should provide high importance to the implementation of Water, Sanitation, and Hygiene (WASH) facilities in schools, incorporating them into wider efforts like the Sarva Shiksha Abhiyan (SSA). This can be achieved by offering incentives to schools that possess properly maintained water, sanitation, and hygiene (WASH) facilities, and by actively involving stakeholders, including the community, school teachers, and student councils. The main barriers hindering the implementation of sustainable WASH practices in government schools in Tamil Nadu include a lack of awareness and understanding about menstruation, inadequate infrastructure, a lack of behavioral change and community involvement, ineffective integration of ICT, a lack of coordination between departments, the need for national standards, unequal access to safe water and sanitation, and the need to institutionalize WASH in schools. In order to promote gender equality and ensure the provision of high-quality education, it is imperative to address these challenges.

**Assessing Socio-Cultural Factors in School WASH Adoption**

Multiple socio-cultural factors impact the adoption and long-term viability of WASH facilities in schools in Tamil Nadu. A study conducted in the state found that over 50% of the student population was comprised of girls, and a substantial portion of them often experienced waterborne diseases, such as diarrhea, due to the lack of functioning bathroom facilities at the school. This discourages girls from regularly attending classes, as parents are unwilling to

force their daughters to go to schools that do not have adequate WASH facilities. To address this issue, a regional organization, supported by WaterAid, aided a school in constructing hygienic facilities that have distinct areas for male and female pupils. In addition, they supplied hydraulic lift water pumps along with a raised storage tank and hand washing facilities.

The intervention involved setting up a school sanitation committee and kid cabinet, which played a vital role in promoting hygiene. Children were given the responsibility of supervising facilities and spreading crucial hygiene messages to their peers. The individuals were tasked with the responsibility of up keeping and utilizing the recently implemented sanitation and hygiene facilities appropriately, while also guaranteeing the cleanliness and safety of the school premises. As a result, this intervention has resulted in a rise in school attendance and a decline in absenteeism due to illness. The findings underscore the need of including the community, specifically parents and children, in the execution and maintenance of WASH facilities in schools. Socio-cultural factors, such as gender norms and attitudes towards education, specifically in relation to girls, can significantly impact the implementation and sustained effectiveness of WASH programs in schools. Through community engagement and the empowerment of children, we can effectively tackle these issues and attain improved results in water, sanitation, and hygiene (WASH). This strategy is advantageous for improving educational attainment, particularly among female students.

**Evaluating WASH Policy and Financial Impact**

The WASH policies of India are designed to tackle concerns pertaining to Water, Sanitation, and Hygiene. Nationally, efforts such as the Swachh Bharat Mission have played a crucial role in promoting sanitation and hygiene practices, as well as implementing plans that address water supply and conservation. Tamil Nadu implements state-specific programs that supplement national initiatives. The objective of the Tamil Nadu Urban Sanitation Policy is to enhance the urban sanitation infrastructure and services, with a focus on promoting community involvement and implementing sustainable practices. In addition, initiatives such as the Tamil Nadu Urban Flagship Program give special importance to interventions related to water supply, sanitation, and hygiene in urban areas. Both national and state-level policies prioritize the significance of collaborations between government agencies, local communities, and other stakeholders to accomplish comprehensive WASH objectives. These policies generally encompass plans for the improvement of infrastructure, communication tactics to promote behavior change, and monitoring tools to measure progress and ensure responsibility. The existing regulatory frameworks and financial investments have shown minimal efficacy in enhancing water, sanitation, and hygiene (WASH) infrastructure in numerous developing nations. The UNICEF guidance on building WASH finance solutions emphasizes various significant obstacles: Several countries do not have a thorough assessment of the overall expenses needed to meet their national Water, Sanitation, and Hygiene (WASH) goals

Insufficient resources can be challenging to mobilize without a comprehensive awareness of the financial requirements. The tracking and mapping of financial flows to the WASH sector are frequently inadequate. Most countries lack WASH accounts that routinely gather and evaluate expenditure data. This hinders the ability to accurately identify areas where money is lacking and allocate expenditures in a focused manner. The allotments from public finances to WASH are often insufficient and uncertain. The level of participation in budget procedures and the standard of budget submissions by WASH ministries require improvement. Contributions from users through tariffs typically only cover a small portion of the expenses associated with operating and maintaining a system, and do not even come close to covering the costs of capital investments. The billing and collection systems exhibit deficiencies, while the tariff structures lack effective design to achieve a balance between affordability and cost recovery. Transfers from external sources, such as development assistance, are significant in numerous nations, although they do not always correspond effectively with national priorities. The ability to effectively employ these monies is constrained by limited absorptive capacity. WASH service providers sometimes struggle to get repayable money, such as loans and bonds, because they lack creditworthiness and viable projects that banks are willing to invest in. The development of enabling settings for public-private cooperation is inadequate.

In order to enhance the efficiency of WASH investments, the UNICEF guidance suggests the development of a comprehensive WASH financial plan. This is the process of calculating the expenses required to meet the national WASH objectives, evaluating the present and anticipated financial inflows from tariffs, taxes, and transfers, finding strategies to decrease costs and enhance revenues, and proposing a set of policies to bridge the funding shortfall. The formulation of a WASH funding plan involves the collaboration of government, service providers, civil society, and development partners to ensure that investments are in line with national priorities. It promotes the governance of the sector, improves the quality of policies and expenditures, and increases the sector's reputation to get new sources of money. Although there has been some improvement in current policy frameworks and expenditures, there are still significant gaps in financing WASH infrastructure. To expedite development and attain widespread availability of secure and sustainable services, it is imperative to adopt a more strategic, evidence-based and collaborative approach by using WASH funding initiatives.

**Innovative WASH Solutions in Government School**

Tamil Nadu's government schools have effectively tackled WASH-related obstacles through innovative approaches and the implementation of exemplary methods. Many schools in Thiruvallur and Vellore districts have demonstrated outstanding performance in WASH (Water, Sanitation, and Hygiene) practices, highlighting the significant impact of effective management in achieving spectacular results. These schools have consistently upheld high standards of cleanliness and hygiene and have been acknowledged with the Swachh Vidyalaya Puraskar (SVP). The schools have employed behavior-oriented activities to foster awareness regarding the need of sanitation and hygiene. These efforts have involved parents and the wider community, resulting in beneficial and effective outcomes. Community-driven efforts have successfully generated and disseminated information about various social welfare programs, fostering harmony among communities.

The adoption of Information and Communication Technology (ICT) has been made easier by the execution of the Swachh Bharat Abhiyan (SBA) and Swachh Bharat Swachh Vidyalay (SBSV) projects. Although the use of ICT in the process is advantageous progress, there is a deficiency in comprehending its exact purpose. These programs have prioritized the promotion of infrastructure development and the provision of incentives to schools for the adoption of hygienic practices. Sanitation is the responsibility of each state, and each state encounters unique difficulties in dealing with this issue. For example, in Tamil Nadu, despite having enough infrastructures, the dropout rate for girls remains high, partially because of insufficient understanding about menstrual hygiene. In order to tackle this issue, it is essential to implement initiatives that prioritize the development of knowledge and comprehension, as well as the modification of behaviors. This will guarantee that the outcomes are more significant and long-lasting in the future. The government should enact initiatives that organically foster residents' incorporation of sanitation and hygiene practices into their daily regimens. It is imperative to arrange subsequent training sessions that actively involve individuals and communities in acquiring the necessary skills to efficiently utilize bathrooms and similar facilities. The state must ensure the establishment of governing bodies or committees in each district those benefits from the system. In the age of digitization, where communication has grown easier and more effective, it is crucial to prioritize the progress of information.

**UNICEF's vision for WASH**

UNICEF's WASH (Water, Sanitation, and Hygiene) goal focuses on ensuring that everyone, especially those in regions like Tamil Nadu, has access to clean water and proper sanitation, since it is their fundamental human right. The objective of the WASH Strategy is to attain widespread and fair availability of clean and reasonably priced drinking water by 2030, and to guarantee access to sufficient and just sanitation and hygiene practices while eradicating open defecation. The strategy places particular emphasis on addressing the requirements of women, girls, and vulnerable people. These objectives are in accordance with the specific targets outlined in Sustainable Development Goal (SDG) 6 for water, sanitation, and hygiene. They contribute to the overall 2030 Agenda for Sustainable Development, which is of utmost importance for the well-being of children. It is crucial to acknowledge that achieving sustainable development, particularly in regions like Tamil Nadu, requires comprehensive collaboration from all stakeholders to address various aspects of SDG 6, such as water quality, efficiency, resource

management, and ecosystem protection. Without these collective efforts, the provision of safe drinking water and sanitation services may be compromised.

**Community Participation in Sustainable WASH Practices**

Community involvement is crucial for enhancing the long-term sustainability of water, sanitation, and hygiene (WASH) practices in Tamil Nadu. The success of initiatives such as the Swachh Bharat Abhiyan (SBA) and Swachh Bharat Swachh Vidyalay (SBSV) projects in the state can be attributed to the active involvement of local communities. The focus of these initiatives has been on developing infrastructure and offering incentives to schools to encourage the adoption of hygienic practices, leading to significant improvements in water, sanitation, and hygiene (WASH) standards. The implementation of the SBA and SBSV programs has facilitated the use of Information and Communication Technology (ICT) to spread awareness about the importance of sanitation and hygiene. This method has demonstrated significant effectiveness in actively engaging communities and cultivating a strong feeling of ownership among them. The programs have also emphasized the importance of community-driven initiatives, leading to the creation of WASH committees within communities to supervise the operation and maintenance of built toilets.

The SANTOLIC initiative, aimed at improving sanitation and hygiene habits among the Irular indigenous population, also known as the Irula or Iruliga, represents the importance of community engagement. The Irula people are a Dravidian ethnic group and a scheduled tribe in India. The program involves the installation of residential bathrooms for 50 households and the promotion of the CLTS concept (Community-Led Total Sanitation) through campaigns and informative activities. The effort has successfully increased awareness and disseminated information to encourage sustainability, so assuring the optimal exploitation of the built toilets. The School Led Total Sanitation (SLTS) initiative exemplifies the importance of community engagement in enhancing the long-term sustainability of water, sanitation, and hygiene (WASH) practices. This campaign has been effectively executed in other states, including Tamil Nadu. The program involves instructing young individuals on the importance of sanitation and hygiene, leading to a change in behavior and the adoption of respectable WASH practices. Community involvement is essential for enhancing the long-term sustainability of water, sanitation, and hygiene (WASH) practices in Tamil Nadu. The active involvement of local communities has been important in the success of initiatives such as the Swachh Bharat Abhiyan (SBA) and Swachh Bharat Swachh Vidyalay (SBSV) schemes, the SANTOLIC program, and the School Led Total Sanitation (SLTS) program. These endeavors have cultivated a feeling of possession among communities, ensuring the successful implementation and upkeep of Water, Sanitation, and Hygiene (WASH) practices.

**Integrating WASH Education: Impact on Behavior**.

The incorporation of Water, Sanitation, and Hygiene (WASH) teaching into the school curriculum in Tamil Nadu has been a noteworthy measure in fostering healthy habits among students and staff. Education on cleanliness is crucial to ensure that students cultivate proper hygiene practices, which are vital for their general health and well-being. The WASH education program is supposed to be all-encompassing, addressing multiple facets like hand hygiene, toilet utilization, and menstrual hygiene. The program is executed using a range of techniques, such as interactive activities, instructional games, and participatory exercises. The WASH education program has effectively impacted student behavior, specifically in relation to hand hygiene habits. Students are instructed on the significance of practicing proper hand hygiene, particularly after using the restroom and prior to consuming food. Adhering to this practice is essential for reducing the transmission of diseases and promoting optimal health. The curriculum also highlights the necessity of appropriate toilet utilization and upkeep, guaranteeing that kids comprehend the significance of using toilets for their designated function. The WASH education program has additionally exerted a beneficial influence on staff conduct. Educators and other school personnel undergo training to encourage proper hygiene habits among children and to guarantee that the school premises are sanitary and well cared for. This include overseeing daily WASH (Water, Sanitation, and Hygiene) activities, assuring consistent availability of soap and water, and including WASH activities into teacher's duties and performance assessments. Incorporating WASH instruction within the school curriculum has also resulted in heightened community engagement. Parents and

community members are urged to engage in WASH-related activities, such as building WASH facilities at home and advocating for appropriate hygiene practices. Community engagement is essential for the long-term viability of the WASH education program and the widespread adoption of proper hygiene practices, both within schools and households. Incorporating WASH education into the school curriculum in Tamil Nadu has been a noteworthy measure in fostering hygienic behaviors among students and staff. The program has effectively influenced student behavior, specifically in regards to hand washing procedures, and has also positively affected professional conduct. The program's focus on community engagement has guaranteed the adoption of proper hygiene protocols, not just in schools but also inside households, resulting in a healthier and more sanitary environment for everyone.

## FINDINGS OF THE STUDY

1. **Limited Access to Safe Drinking Water**: Only 65% of schools had access to safe drinking water, and 15% of students reported having access to water only occasionally.
2. **Poor Maintenance of WASH Infrastructure**: The study found that the maintenance of WASH infrastructure was inadequate, with 27.7% of students reporting that soap was not offered daily in classrooms.
3. **Inadequate Toilet Facilities**: Half of the students reported that the water in school toilets was unsafe to drink due to poor odor, taste, or color.
4. **Climate Change Impacts**: Climate change-related factors, such as water scarcity and extreme weather events, exacerbate existing challenges in maintaining sustainable WASH infrastructure.
5. **Inadequate Hand washing Facilities**: Despite effective regulations during COVID-19, 27.7% of students reported that soap was not offered daily in classrooms.
6. **Limited Availability of WASH Services**: The study found that the availability of WASH services was limited, with 15% of students reporting that they had access to water only occasionally.
7. **Inadequate Education and Practices**: The study highlighted the importance of education and practices in promoting sustainable WASH practices, but found that these were often inadequate.
8. **Community Involvement Crucial**: The study emphasized the need for community involvement in ensuring the sustainability of WASH practices in schools.
9. **Cultural and Social Norms**: Cultural and social norms may influence attitudes towards hygiene, making it challenging to introduce and sustain behavior change initiatives.
10. **Inadequate Training and Capacity Building:** Inadequate training and capacity-building programs for school staff hinder their ability to effectively manage WASH facilities and educate students on proper practices.
11. **Technological Solutions**: Leveraging technology, such as mobile apps for monitoring and reporting WASH indicators, can enhance data-driven decision-making and facilitate targeted interventions in Tamil Nadu's government schools.
12. **Collaboration Essential**: Collaboration between local authorities, school administration, and parents was seen as essential for meeting the basic needs of WASH in schools.

## CONCLUSION

The effort to implement sustainable Water, Sanitation, and Hygiene (WASH) practices in government schools in Tamil Nadu has encountered challenges and numerous opportunities. Despite facing formidable obstacles such as inadequate infrastructure, limited funding, and bureaucratic limitations, the state has made significant strides in promoting sustainable WASH practices. Tamil Nadu has successfully addressed these challenges by introducing innovative programs and engaging in partnerships with various stakeholders, including non-governmental organizations and community leaders. However, it is essential for government officials to uphold their commitment and financial backing to ensure the sustained feasibility of these programs. The lessons learned from Tamil Nadu's experience emphasize the importance of implementing holistic methods that integrate education, behavior modification initiatives, and infrastructural improvement. In order to ensure long-term success, it is crucial to encourage community engagement and accountability, and to employ technology for the purpose of monitoring and

evaluating. Despite the difficulties that lie ahead, there are ample opportunities to improve water, sanitation, and hygiene (WASH) standards in government schools. Tamil Nadu and other regions have the potential to establish a future in which every school possesses reliable and environmentally-friendly WASH facilities by learning from previous experiences, adopting new ideas, and fostering collaboration. This would ensure a more salubrious and affluent atmosphere for all pupils.

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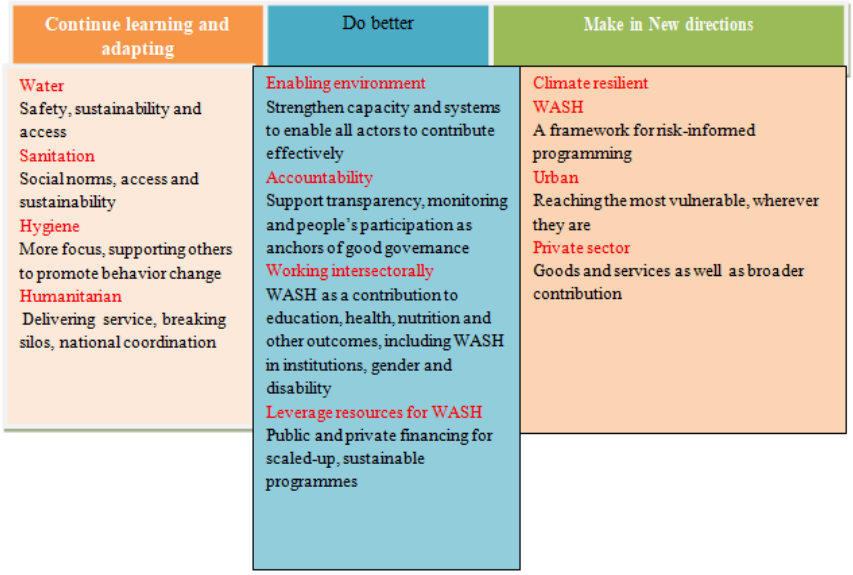


Source: UNICEF. (2016) *Strategy for water, sanitation and hygiene 2016–2030*. Programme Division. New York, NY

**Figure 1. UNICEF's Strategy for WASH (2016-2030)**

**Source:** Source: UNICEF. (2016) *Strategy for water, sanitation and hygiene 2016–2030*. Programme Division. New York, NY

**Figure 2: Context-specific Sanitation Programming**

**Source:** Source: UNICEF. (2016) *Strategy for water, sanitation and hygiene 2016–2030*. Programme Division. New York, NY

**Figure 3: Components of SDG 6: Ensure Availability and Sustainable Management of Water and Sanitation for All**

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**RESEARCH ARTICLE**

# A Cross - Sectional Research on Women with Irregular Menstruation and Obesity in Assam, Northeast India

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## ABSTRACT

Obesity has a strong correlation with menstrual irregularity. Women who are obese often experience oligomenorrhea, amenorrhea, or irregular periods. Moreover, it also increases the risk of developing gynecological diseases, including infertility and menstrual dysfunction. However, it is important to note that a variety of sex hormones also play a crucial role in these disorders. to examine the connection between irregularity of menstruation and obesity among the adult women of Assam. Between February 2022 and August 2022, 100 women, aged 18 to 42 years, who often attended an outpatient obesity clinic at Pratiksha Hospital in Guwahati were the subjects of a cross-sectional research. The women's current height (m) and weight (kg) were measured. These measures were used to compute the body mass index (BMI) and waist-to-hip ratio (WHR). Women's BMIs are used to calculate obesity. A digital sphygmomanometer was used to record the blood pressure. Patients were asked to document menstrual cycle aspects for three months. A range of biological indicators were also looked at. Out of 100 women, women with regular menstrual cycles are found to be 66 (66.00%), women with oligomenorrhea are found to be 22 (22.00%), and women with amenorrhea are found to be 12 (12.00%). The results found that 34 (34.00%) women have had menstrual irregularities. The average age of women with regular menstrual cycles (66), oligomenorrhea (22) and amenorrhea (12) is found to be (29.82±5.63), (28.86±5.87), and (30.08±5.81), respectively. The study shows an association of obesity with ologomenorrhea and amenorrhea.

**Keywords:** obesity, oligomeorrhea, amenorrhea, menstrual irregularity.

## INTRODUCTION

Obesity has an adverse effect on public health and is becoming more common in both developed and developing nations[1-4]. In the modern world, obesity poses a greater hazard to public health than communicable illnesses[3-6]. The WHO reports that between 1980 and 2014, the prevalence of obesity more than quadrupled globally[7]. Studies on migration and comparisons between rural and urban areas show that industrialization has increased the incidence of obesity; in several countries, 50% of people are overweight or obese[8-12]. Obesity received little attention until recently in India, the most populous country, where undernutrition has been the main public health problem for the past few decades [1,13,14]. According to growing data, childhood and adult obesity rates are rising[5,15-18]. In addition to being linked to a number of non-communicable illnesses, such as diabetes, cancer, hypertension, and cardiovascular disorders, obesity is a major risk factor for mortality and disability worldwide[9-12,19-21]. Because of biological differences, females are more likely to be fat[22,23]. Obese women experience menstruation problems, PCOS and infertility [24-29]. Several studies have clearly shown that obese women with PCOS are more likely to experience irregular menstrual periods[26-31]. Although several sex hormones also play a significant part in these problems, obesity significantly raises the chance of developing gynecological diseases, such as menstrual dysfunction and infertility [32-35]. Gynecological issues encompass a broad spectrum of irregularities, such as irregular menstrual cycles, prolonged menstrual cycles, and blood loss throughout the menstrual cycle. Among obese women, irregular menstrual cycles are more common[34,36,37,38]. Additionally, a number of studies have shown a connection between women's menstrual cycle disruption and endometrial, breast, and cardiovascular cancers[28,39-42]. According to Tang *et al.*[43] (2020), women who are overweight have a much higher chance of having endometrial hyperplasia.

Abnormal menstrual periods are associated with obesity. A correlation exists between body mass index (BMI) and irregular menstrual cycles, as reported by Wei *et al.*[44]. According to Wei *et al.*[44], oligomenorrhea and irregular menstruation are more common in people with BMIs over 25 kg/m$^2$. According to a number of studies [28,29,38,45-47], obese women experience a larger percentage of abnormal menstrual cycles than the general population. Weight loss improves women's cycles. Obesity and menstruation are fundamentally related because fat cells contain cholesterol molecules that can be converted into "estrone", a weak form of estrogen. Women carrying additional fat cells have "little estrogen-making factories", which have an estrogenic influence on their glands. The excess estrogen may lead to irregular periods or bleeding[34,48]. Therefore, we examined the relationship between obesity levels and abnormal menstrual cycle length in a group of obese women, as well as the incidence of amenorrhea and oligomenorrhea, as a means of understanding the impact of obesity on reproduction.

## MATERIALS AND METHODS

We conducted a cross-sectional study between February and August 2022, involving 100 women from the age range of 18 to 42 years who attended an obesity clinic at Pratiksha Hospital in Guwahati. Women with PCOS on ultrasonography and those who claimed to be pregnant or to have experienced menstrual problems in the past were not included. We measured the subjects' current body weight (kg), height (cm). The narrowest point above the hip served as the measurement point for the waist, while the highest point of the gluteus protuberance served as the measurement point for the hip. Waist-to-hip ratio (WHR) and body mass index (BMI) was calculated from these measurements. Obesity is calculated through the BMI of the women. Blood pressure was recorded by using a digital sphygmomanometer. For three months, women recorded the length of their menstrual cycles. Women were diagnosed with oligomenorrhea if their periods lasted between 36 and 90 days, amenorrhea if they hadn't had one in more than 90 days, and normal if their cycles lasted between 25 and 35 days.Among the biochemical indicators analyzed were biological parameters such as serum insulin, total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density lipoprotein, and total cholesterol.

**Statistical analysis**

The Statistical Package for Social Sciences (Version 16.0) was used to analyze the data after they were imported into Microsoft Excel. Means and standard deviations were used to express continuous variables, and percentages were used to express categorical variables. Comparing anthropometric traits and biological markers between women with regular menstrual periods, oligomenorrhea and amenorrhea was done using analysis of variance. Spearman correlation coefficient was applied to see the strength of correlation of obesity with menstrual irregularities based on age and the anthropometric parameters. A p-value (<0.05) was considered to be statistically significant.

## RESULTS

Table I displays the anthropometric characteristics of obese women with and without regular menstrual periods. Of 100 women, 66 (66.00%) had normal menstrual cycles, 22 (22.00%) had oligomenorrhea, and 12 (12.00%) had amenorrhea. According to the findings, 34 women (34.0%) had irregular menstruation. The mean age of women who have oligomenorrhea (age 22), amenorrhoea (12), and normal menstrual cycle (age 66) is (29.82±5.63), (28.86±5.87), and (30.08±5.81) years, respectively. Women with oligomenorrhea (79.84±6.60) (kg), normal menstrual cycle (76.15±7.19) (kg), and amenorrhoea (80.83±8.86) (kg) had higher average weights (kg). Similarly, women with amenorrhoea (92.88±6.84) (cm), oligomenorrhea (88.81±6.02) (cm), and normal menstrual cycles (80.65±4.62) (cm) had the largest average waist circumference (WC). The same is true for hip circumference (cm), where women with normal menstrual cycles (91.54±4.94) (cm), oligomenorrhea (94.42±8.37) (cm), and amenorrhoea (98.58±9.95) (cm) had the greatest average HC. Women who experienced irregular menstruation, such as oligomenorrhea (33.69±1.32) and amenorrhoea (34.92±1.32), had higher body mass indices (BMIs). The waist-hip ratio (WHR) is estimated to be 0.88±0.03 (kg/m$^2$) in women with regular menstrual cycles and higher in those with oligomenorrhea (0.89±0.06) and amenorrhoea (0.89±0.07). Women with amenorrhoea had the greatest diastolic blood pressure (mm/hg) (87.58±15.58) (mm/hg), whereas women with regular menstrual cycles have normal systolic blood pressure (mm/hg) (120.39±11.69) (mm/hg). The overall blood pressure of women with regular menstrual cycles was 120.39±11.69/84.69±8.93 (mm/hg), which is considered normal. The age of menarche, measured in years, is highest in women with normal menstrual cycles (12.59±1.21), lowest in amenorrhoea (11.58±1.38), and lowest in oligomenorrhea (12.27±1.42) (years). No discernible differences are observed in age, blood pressure, or BMI. Additionally, the results based on age at menarche, weight (kg), WC, HC, and WHR indicated statistically significant outcomes.

According to Table II, women with regular or irregular menstrual periods and obesity are associated with different biochemical indicators. Therefore, oligomenorrhea (101.41±21.09) (mg/dL) and amenorrhoea (104.67±18.33) (mg/dL) were associated with greater levels of glucose intolerance in women with irregular menstruation. When comparing women with normal menstrual cycles (118.21±20.04) to those with oligomenorrhea (124.42±32.07) (mg/dL), it was shown that triglycerides were considerably greater in women with amenorrhoea (135.75±36.17) (mg/dL). Women with irregular menstrual cycles had higher basal insulin concentrations. This is particularly true for those with amenorrhoea (22.06±10.86) (μU/dL), oligomenorrhea (17.10±8.97) (μU/dL), and normal menstrual periods (14.69±6.43) (μU/dL). There was no difference in HDL, LDL, or total cholesterol levels according to menstrual irregularity. Triglycerides, basal insulin levels, and glucose intolerance all exhibit statistically significant results. Table III examined the degree to which menstrual abnormalities and obesity are correlated. The results indicated a direct relationship between WHR and amenorrhoea (*rho*=0.04950) and oligomenorrhea (*rho*=0.04939). Additionally, there is a strong positive link between blood pressure and irregular menstruation, such as amenorrhoea and oligomenorrhea.

## DISCUSSION

According to research, irregular menstruation is highly correlated with obesity. More specifically, class 2 obese women (BMI 30.0-39.9 kg/m$^2$) are more likely to experience oligomenorrhea and amenorrhea. A study conducted by Souza *et al*. [49]assessed fifty-seven women who had class III obesity, or morbid obesity (≥40 kg/m$^2$). This type of obesity can lead to menstrual dysfunctions such as amenorrhea or oligomenorrhea[26-29,38,47]. WHR and BMI are

anthropometric assessments that indicate how much body fat a person has. WHR is more suggestive of abdominal fat deposition, which has been linked to diabetes mellitus and hypertension and is more hazardous; an increase in these parameters is a sign of obesity [50-52]. Numerous factors, including hormone flux, underlying medical conditions, and body mass as determined by weight and BMI, have been found to affect menstrual irregularity and flow[50,53-55]. A disrupted hormonal balance may cause aberrant menstrual patterns, which in turn may mediate the risk of hypertension[56,57]. Since androgens can raise blood pressure and help with the etiology of hypertension, they can both help prevent cardiovascular diseases (CVDs) such as hypertension by acting as vasodilators [56-60]. Furthermore, some well-established risk factors for irregular menstruation—such as age, smoking, stress, weight, family history, and pregnancy history—also increase the likelihood that obese women may develop hypertension [61-64]. Additionally, the incidence of hypertension in obese women with and without irregular menstruation was demonstrated in the current study.

A comparison of biochemical markers between obese women with normal menstrual cycles, oligomenorrhea, and amenorrhea is presented in Table II. Obesity is closely linked to menstrual abnormalities and unfavorable hormonal profiles[49,56,57,65]. Research has demonstrated that women with oligomenorrhea or amenorrhoea had much higher blood glucose concentrations; these findings are supported by studies conducted by Shen *et al.*[66]and Klein *et al.*[67]. Results showed that blood glucose levels were associated with irregular menstrual periods and obesity. This finding led us to speculate—a theory previously advanced by others—that obesity, while not the cause of PCOS, may facilitate its phenotypic manifestation in women who are predisposed to the illness by resulting in insulin resistance and hyperinsulinemia[44,68,69,70,71]. In our investigation, women with oligomenorrhea and amenorrhoea had higher insulin levels than women with normal menstrual cycles, a finding previously observed in studies by Shim *et al.*[68], Ko*et al.*[69], Itriyeva[70], and Jalil *et al.*[71]. As a result of hyperinsulinemia, increased androgen synthesis may negatively impact the menstrual cycle and decrease the likelihood of conception[27,72-76]. In the current study, reproductive rates decreased when fat levels rose in response to an increase in insulin disruption. Additionally, Seif *et al.*[77] found that insulin and androgens affect steroid levels in the ovarian stroma, contributing to issues with ovulation and unpredictable menstruation. Moreover, hyperinsulinemia and high lipid levels prevent the production of LH and FSH, which alter menstrual periods [74,78-80].Additionally, the current study demonstrated a favorable relationship between oligomenorrhea and amenorrhoea and blood pressure, weight, and WHR (Table 3). Sheela *et al.*[81]discovered that oligomenorrhea is most commonly associated with a BMI over 25 and that greater obesity grades increase the risk of irregular periods. Additionally, a positive correlation between the menstrual profile and BMI was found[50,82-84]. Oligomenorrhea and other menstrualabnormalities are common in obese women. Similar to the current study, a study Mustaqeem *et al.*[36]found that irregular menstrual periods were experienced by 64.44% of individuals with increased WHR.

## CONCLUSION

This study advances our knowledge of the relationship between obesity and menstrual problems in women. Retaining a healthy weight helps reduce the likelihood of irregular menstruation and the gynecologic issues associated with obesity.

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**Table I: Anthropometric characteristics of obese women with and without regular menstrual cycles**

| Variables | Regular Menstrual Cycle (n=66) | Oligomenorrhea (n=22) | Amennorrhea (12) | p* value (Analysis of variance) |
|---|---|---|---|---|
| Age (years) | 29.82±5.63 | 28.86±5.87 | 30.08±5.81 | 0.76850 |
| Height (cm) | 154.66±6.45 | 153.84±5.91 | 152.06±6.70 | 0.00004 |
| Weight (kg) | 76.15±7.19 | 79.84±6.60 | 80.83±8.86 | 0.03421 |
| BMI (kg/m$^2$) | 31.77±1.50 | 33.69±1.32 | 34.92±1.32 | 0.42630 |
| Waist circumference (cm) | 80.65±4.62 | 88.81±6.02 | 92.88±6.84 | < 0.00001 |
| Hip circumference (cm) | 91.54±4.94 | 94.42±8.37 | 98.58±9.95 | 0.00285 |
| Waist Hip Ratio (kg/m$^2$) | 0.88±0.03 | 0.89±0.06 | 0.89±0.07 | 0.55703 |
| Blood Pressure Diastolic (mm Hg) | 84.69±8.93 | 82.05±10.24 | 87.58±15.58 | 0.31999 |
| Blood Pressure Systolic (mm Hg) | 120.39±11.69 | 117.59±15.83 | 119.08±18.52 | 0.70738 |
| Age at Menarche (Years) | 12.59±1.21 | 12.27±1.42 | 11.58±1.38 | 0.00452 |

**Table II. : Biochemical markers of obese women with and without regular menstrual cycles**

| Variables | Regular Menstrual Cycle (n=66) | Oligomenorrhea (n=22) | Amennorrhea (12) | *p* value (Analysis of variance) |
|---|---|---|---|---|
| Glucose (mg/dL) | 91.53±17.40 | 101.41±21.09 | 104.67±18.33 | 0.02236 |
| Triglycerides (mg/dL) | 118.21±20.04 | 124.42±32.07 | 135.75±36.17 | 0.04609 |
| HDL Cholesterol (mg/dL) | 54.14±8.84 | 50.55±10.29 | 55.08±12.24 | 0.27761 |
| LDL Cholesterol (mg/dL) | 120.42±4.94 | 117.73±20.94 | 124.83±28.40 | 0.65130 |
| Total Cholesterol (mg/dL) | 180.92±22.06 | 178.95±23.93 | 182.58±33.12 | 0.90983 |
| Insulin (μU/dL) | 14.69±6.43 | 17.10±8.97 | 22.06±10.86 | 0.01073 |

**Table III. Spearman correlation coefficient of obesity with menstrual irregularities**

| Variables | Oligomenorrhea (22) | | Amenorrhea (12) | |
|---|---|---|---|---|
| | **R-value** | **p-value** | **R-value** | **p-value** |
| Weight (kg) | 0.67988 | 0.00050 | 0.83363 | 0.00075 |
| Waist circumference(cm) | 0.06563 | 0.77168 | 0.75657 | 0.00440 |
| Hip circumference(cm) | 0.04980 | 0.82580 | 0.75657 | 0.00440 |
| WHR | 0.42374 | 0.04939 | 0.57701 | 0.04950 |
| Diastolic BP (mm of Hg) | 0.06423 | 0.77642 | 0.74211 | 0.00572 |
| Systolic BP (mm of Hg) | 0.08871 | 0.69462 | 0.77193 | 0.00327 |

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**RESEARCH ARTICLE**

# Influence of Poultry Manure Compost and Foliar Application of Seaweed Extract on the Productivity and Profitability of Finger Millet (*Eleusine coracana* L.)

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## ABSTRACT

Field experiment was conducted at Puliyanthoppu village in Krishnagiri district, Tamil Nadu, from December 2019 to April 2020 to study the Influence of poultry manure compost and foliar application of seaweed extract on the productivity and profitability of finger millet (*Eleusine coracana* L.) under irrigated conditions. The experiment followed a split-plot design with three replications. According to the experimental results, the application of enriched poultry manure compost @ 750 kg ha$^{-1}$ combined with foliar spray of seaweed extract @ 0.3% significantly recorded growth characteristics, yield attributes, and grain yield. This treatment also registered higher gross return of Rs. 91,019 ha$^{-1}$, net return of Rs. 53,777 ha$^{-1}$, and B:C ratio of 2.44. Conversely, the least growth and yield attributes, as well as the lowest gross return, net return, and B:C ratio, were observed with the application of enriched FYM @ 750 kg ha$^{-1}$ along with water spray.

**Key words:** Enriched poultry manure compost, Seaweed extract, grain yield, gross return, net return and BCR

## INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaertn.) Ranks as the third most important millet in India, following sorghum and pearl millet. It stands out among major food grains due to its high nutritional value, particularly in protein and minerals such as calcium and iron, offering 8–10 times more calcium than wheat or rice. Its slow digestibility makes it an excellent food crop for pregnant women and individuals with diabetes. Finger millet accounts for about 85% of total millet production in India, covering 11.9 lakh hectares and yielding 19.8 lakh tonnes with a productivity of 1662 kg per hectare. In Tamil Nadu, it is grown on 0.78 lakh hectares, producing 2.56 lakh tonnes with a productivity of

1966 kg per hectare. Nutrient management is a crucial factor in enhancing the productivity of finger millet. While the application of chemical fertilizers can significantly boost crop yields, it often has a detrimental effect on soil properties. Therefore, an integrated approach to nutrient management is essential to prevent nutrient depletion, maintain soil fertility, and sustain crop productivity. Organic amendments, such as farmyard manure, poultry manure, and pressmud compost, positively impact soil fertility, improving soil condition and plant growth.

Poultry manure holds a prominent position due to its higher nutrient content compared to other manures. (Adeleye *et al.,* 2010). The application of farmyard manure (FYM) enhances soil fertility by improving its physical condition and water holding capacity. FYM not only supplies macronutrients but also meets micronutrient requirements, thereby improving soil health (Jadhao *et al.,* 2019). Pressmud is a valuable resource of plant nutrients and improves the physical, chemical, and biological properties of the soil, leading to increased crop yields (Ramesh, 2018). Foliar application of liquid organic manures influences influences a wide range of physiological parameters, including altered plant structure, assimilate partitioning, increased nutrient uptake, improved nitrogen metabolism, accelerated blooming, grain filling, and enhanced assimilate mobilization to designated sinks (Sharma Sardana and Sukhvinder Singh, 2013).

To provide additional nutrients, developing liquid organic biostimulants such as seaweed extract and humic acid for foliar application is essential. These biostimulants can supply necessary nutrients during critical periods of crop growth and possess insecticidal or fungicidal properties. Seaweed extracts are marketed as liquid fertilizers and bio-stimulants due to their high content of growth regulators like cytokinins and gibberellins. Alongside these growth regulators, seaweed extracts provide a wide range of macro and micronutrients essential for optimal plant growth and development (Khan *et al*., 2009). Humic compounds enhance soil structure, promote the growth of soil microbes, increase the cation exchange capacity of the soil, and indirectly benefit plant roots by supplying essential macro and micronutrients, thus improving soil fertility (Guo *et al*., 2009). While there is extensive research on the use of organic manures and foliar application of biostimulants in finger millet individually, studies on the combined application of organic manures—particularly enriched organic compost—and liquid organic manures through foliar application in irrigated finger millet are limited. Therefore, this study aimed to develop integrated nutrient management strategies for irrigated finger millet that would be profitable, productive, and sustainable for farmers in the Krishnagiri District of Tamil Nadu, India.

## MATERIALS AND METHODS

**Experimental site**

Field experiment was performed in a farmer's field at Puliyanthoppu village, Krishnagiri District, Tamil Nadu from December 2019 to April 2020 (Marghazipattam). The field site was geographically located at an altitude of 492 m above mean sea level, at $11^0$12 to $12^0$49′ North latitude and $77^0$27 to $78^0$38′ East longitude. The weather at Puliyanthoppu village was dry, with scanty rainfall except during the monsoon season. The mean annual rainfall received was 830 mm. During the farming season, the maximum temperature is 34.24 $^0$ C, the minimum temperature is 22.28 $^0$ C and the relative humidity is 75.50%. The experimental soil has a clay loam texture, pH of 7.1, organic carbon content of 0.58%, and contains nitrogen (214.96 kg/ha), phosphorus (21.37 kg/ha), and potassium (275.28 kg/ha).

## METHODOLOGY

The experiment comprised of three main plot treatments *viz*., $M_1$- Enriched FYM @ 750 kg $ha^{-1}$, $M_2$- Enriched pressmud compost @ 750 kg $ha^{-1}$, $M_3$- Enriched Poultry manure compost @ 750 kg $ha^{-1}$ and five subplot treatments namely, $S_1$- Control (Water spray), $S_2$- Foliar spray of humic acid @ 0.3%, $S_3$-Foliar spray of panchagavya @ 3%, $S_4$-Foliar spray of vermiwash @ 5%, $S_5$-Foliar spray of seaweed extract 0.3 % (All the foliar sprays were given at 20,40 and 60 DAT). The experiment with different treatments was tested in the field in a split-plot design with three

replications. In this study, composted manures such as FYM, pressmud, and poultry manure were used. Enriched FYM compost was prepared using the heap method with the following procedure: 750 kg of FYM (dry-weight basis) was thoroughly mixed with single superphosphate (187.5 kg $ha^{-1}$), Azospirillum (10 kg), and Phosphobacteria (10 kg). The mixture was kept in a shaded area with 60% moisture. After two months, the enriched FYM compost was ready and applied to the designated plots according to the treatment schedule prior to transplanting. This same method was used to prepare enriched compost from poultry manure and pressmud. Promising CO 15 (120 days) finger millet variety was used in the study. Two seedlings per hill were transplanted at the age of 18 days and the spacing adopted was 30 x 10 cm. A fertilizer schedule of 60 kg N, 30 kg P2O5, and 30 kg K2O per hectare was implemented. Nitrogen, phosphorus, and potassium were supplied through urea, single superphosphate, and muriate of potash, respectively. All other improved recommended practices outlined in the Crop Production Guide (2019) were followed for cultivating finger millet.

**Data collection**

Five plants were randomly chosen from each net plot area and tagged for biometric observation at various crop growth phases.

**Plant height**

The plant height was measured from the ground level to the tip of the top most leaf/ ear head at harvest stage.

**Root length and root volume**

The entire finger millet plant was uprooted at flowering stage before harvesting and roots were washed. The root length was measured from the base of the plant to the root tip and expressed in cm. After measuring root length, the roots were separated from the plants and were washed with water and the root volume measured by water displacement method (Karthikeyan, 1999) and expressed in cubic centimetre (cc).

**Crop growth rate (CGR)**

The CGR explains the dry matter accumulated per unit land area per unit time, expressed as g $m^{-2}$ $day^{-1}$. It was calculated by using the following formula as suggested by Watson (1958).

$$CGR = \frac{W_2 - W_1}{(t_2 - t_1)\, s} \; g / m^2 / day$$

Where,

$W_1$, $W_2$ - Plant dry weight (g) at time $t_1$, $t_2$, S – land area ($m^2$)

**Number of fingers ear head$^{-1}$**

At the time of harvest, five matured ear heads were selected at random from the representative plant samples. Number of fingers in each ear head was counted and the mean value was expressed as number of fingers ear $head^{-1}$.

**Length of ear head (cm)**

Ear head length was measured from the point of rear to the tip of the ear head obtained from five primary ear head of the tagged plants and the mean length for ear head was calculated and expressed in cm.

**Grain yield**

Harvesting was done in each plot separately from the net plot area and grains were separated, cleaned and grain yield was recorded plot wise at 14 per cent moisture content. The yield was computed to kg $ha^{-1}$.

**Economic analysis**

The economic parameters such as gross income, net income and benefit cost ratio for all the treatments were worked out based on the prevailing market price. The net income was worked out for different treatments by subtracting the cost of cultivation from gross income. The benefit cost ratio was calculated by using the following formula.

$$BCR = \frac{\text{Gross income (Rs.ha}^{-1}\text{)}}{\text{Cost of cultivation (Rs.ha}^{-1}\text{)}}$$

**Statistical analysis**

The data on the numerous characters evaluated throughout the research study were statistically analyzed. To make the statistical inferences, the critical differences were calculated at a 0.05 per cent probability level (Panse and Sukhatme,1989).

## RESULT AND DISCUSSION

**Growth attributes**

Among different growth attributing characters, enriched poultry manure compost @ 750 kg ha$^{-1}$($M_3$) registered significantly maximum values of plant height of 103.64 cm, root length of 24.93 cm, root volume of 10.82 cc, LAI of 5.71 and crop growth rate of 11.24 g m$^{-2}$ d$^{-1}$ at 30-60 DAT and 8.53 g m$^{-2}$ d$^{-1}$ at 60 - harvest stage (Table 1.). These could be attributed to an adequate supply of nutrients that caused the crops to grow quickly, which in turn increased various metabolic processes, improved the mobilization of synthesized carbohydrates in amino acids and proteins, and consequently promoted cell division and elongation, leading to higher growth attributes. The results are in agreement with the findings of Abdul Nasir *et al*., (2010) and Enujeke (2013).

The least plant height of 92.36 cm, root volume of 7.77cc, root length of 20.56, LAI of 3.83 and crop growth rate of 8.39 g m$^{-2}$ d$^{-1}$ at 30-60 DAT and 6.31 g m$^{-2}$ d$^{-1}$ at 60- harvest were recorded under enriched FYM compost @ 750 kg ha$^{-1}$ ($M_1$). With regard to foliar nutrition, seaweed extract @ 0.3% on 20, 40 and 60 DAT ($S_5$) registered highest plant height of 103.52 cm, root length of 24.92 m, root volume of 10.77 cc, LAI of 5.68 and crop growth rate of 11.18 and 8.29 g m$^{-2}$ d$^{-1}$ at 30-60 DAT and 60 DAT- harvest, respectively. These might be due to availability of plant nutrients and growth regulators in seaweed extract, which helps to enhance the better root development and enzyme activity in the root portion resulting in higher growth attributes registered under this treatment. The results are in concomitance with the findings of Rathore *et al.,* (2009); Liu and Lijun (2011). The least plant height of 88.87 cm, root length of 18.88 cm, root volume of 6.61 cc, LAI of 3.39 and crop growth rate of 8.58 and 6.48 g m$^{-2}$ d$^{-1}$ at 30-60 DAT and 60 DAT- harvest, respectively was recorded under control water spray ($S_1$). Significant interactions were observed between enriched compost and foliar spray. The treatment combination of enriched poultry manure compost @ 750 kg ha$^{-1}$ and seaweed extract @ 0.3% ($M_3S_5$) registered maximum values of plant height, root length, root volume and crop growth rate at 30-60 DAT and 60 DAT- harvest. This could be due to combined effect of composted poultry manure and foliar application of seaweed extract. The least growth characters were recorded under enriched FYM compost @ 750 kg ha$^{-1}$ followed by water spray ($M_1S_1$).

**Yield attributes and yield**

Among different yield attributing characters, enriched poultry manure compost @ 750 kg ha$^{-1}$ ($M_3$) registered significantly higher number of fingers earhead$^{-1}$ of 8.08, length of fingers of 8.98 (Table 2) and grain yield of 3504 kg ha$^{-1}$. These could be due to the poultry compost contains uric acids, which speeds up the release of nutrients and may have resulted in more extensive root development, so that crop can able to absorb more nutrients from the soil, and results in quicker conversion of synthesised photosynthates into protein to form more protoplasm thus led to increased yield attributes and yield. These results were concomitant with the finding of Agbede *et al.,* (2008). The least yield attributes and yield were registered under the treatment enriched FYM compost @ 750 kg ha$^{-1}$ ($M_1$). In respect of foliar nutrition, higher number of fingers ear head$^{-1}$ of 8.05, length of fingers of 8.93cm and grain yield of 3567 kg ha$^{-1}$were obtained with the foliar spray of seaweed extract @ 0.3% on 20, 40 and 60 DAT ($S_5$). The balanced growth pattern may have reduced flower shedding, which on the other hand resulted in a positive source-sink gradient of photosynthates translocation due to growth regulator and also increases the flower production by encouraging timely nutrient supply by foliar spray. Similar observations were reported by Diviya and Kalyani (2016).

The least number of fingers ear head$^{-1}$ of 4.36, length of ear head of 5.21 and grain yield of 2389 kg ha$^{-1}$ were registered with water spray ($S_1$).

The interaction effect between enriched compost and foliar nutrients were found to be significant. Application of enriched poultry manure compost @ 750 kg ha$^{-1}$ followed by foliar spray of seaweed extract @ 0.3% on 20, 40 and 60 DAT ($M_3S_5$) registered the maximum yield attributes *viz.,* number of fingers ear head$^{-1}$, finger length and grain yield. The least yield characters were recorded under enriched FYM compost @ 750 kg ha$^{-1}$ followed by water spray ($M_1S_1$). In respect of harvest index significantly higher values registered under plots received with enriched poultry manure compost @ 750 kg ha$^{-1}$ followed by foliar spray of seaweed extract @ 0.3% on 20, 40 and 60 DAT ($M_3S_5$).

**Economics**

Among the treatment combination of organic manure and foliar nutrition on irrigated finger millet, the maximum gross returns of Rs. 91019 ha$^{-1}$, net returns of Rs. 53777 ha$^{-1}$ and BCR of 2.44 were obtained in plots applied with enriched poultry manure compost @ 750 kg ha$^{-1}$along with foliar spray of seaweed extract @ 0.3% on 20, 40 and 60 DAT ($M_3S_5$) (Table 3). The lowest gross returns of Rs. 50233 ha$^{-1}$, net return of Rs.17041 ha$^{-1}$ and BCR invested 1.51 were recorded in $M_1S_1$ (enriched FYM compost @ 750 kg ha$^{-1}$ along with water spray). Increased profitability in poultry manure compost along with foliar application of seaweed extract could be attributed to finger millet utilized the both below and above the ground available resources very effectively thereby resulted higher grain yield, gross and net returns in irrigated finger millet cultivation (Naidu *et al.*, 2009).

## CONCLUSION

Based on the results of the experiment, it can be concluded that application of enriched poultry manure compost @ 750 kg ha$^{-1}$ followed by foliar spray of 0.3% seaweed extract on 20, 40, and 60 days after transplanting (DAT) to achieve higher productivity and profitability in finger millet cultivation under irrigated conditions.

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**Table 1. Effect poultry compost and foliar application of biostimulants on root length and root volume and growth analysis in finger millet**

| TREAT MENTS | Plant height, root length and root volume | | | Growth analysis | | |
|---|---|---|---|---|---|---|
| | Plant height (cm) | Root length (cm) | Root volume (cc) | LAI | CGR (30-60 DAT) | CGR (60-Harvest) |
| **MAIN PLOTS** | | | | | | |
| **$M_1$** | 92.36 | 20.56 | 7.77 | 3.83 | 9.12 | 6.65 |
| **$M_2$** | 97.12 | 22.37 | 9.07 | 4.77 | 10.12 | 7.37 |
| **$M_3$** | 103.64 | 24.93 | 10.82 | 5.71 | 11.24 | 8.53 |
| **S.Ed.** | 1.14 | 0.28 | 0.12 | 0.06 | 0.12 | 0.09 |
| **C.D (P=0.05)** | 3.27 | 0.79 | 0.35 | 0.19 | 0.36 | 0.27 |
| **SUB PLOTS** | | | | | | |
| **$S_1$** | 88.87 | 18.88 | 6.61 | 3.29 | 8.58 | 6.48 |
| **$S_2$** | 100.16 | 23.67 | 9.92 | 5.20 | 10.70 | 7.86 |
| **$S_3$** | 97.89 | 22.69 | 9.36 | 4.79 | 10.19 | 7.47 |
| **$S_4$** | 98.19 | 22.95 | 9.44 | 4.88 | 10.29 | 7.46 |
| **$S_5$** | 103.52 | 24.92 | 10.77 | 5.68 | 11.18 | 8.29 |
| **S.Ed.** | 0.88 | 0.20 | 0.08 | 0.04 | 0.09 | 0.06 |
| **C.D (P=0.05)** | 1.83 | 0.43 | 0.18 | 0.09 | 0.19 | 0.14 |

**Main plot treatments**: $M_1$- Enriched FYM @ 750 kg $ha^{-1}$,$M_2$- Enriched pressmud compost @ 750 kg $ha^{-1}$, $M_3$- Enriched Poultry manure compost @ 750 kg $ha^{-1}$

**Sub plot treatments**: $S_1$- Control (Water spray), $S_2$- Foliar spray of humic acid @ 0.3%, $S_3$-Foliar spray of panchagavya @ 3%, $S_4$- Foliar spray of vermiwash @ 5%, $S_5$-Foliar spray of seaweed extract (All the foliar sprays were given at 20,40 and 60 DAT).

**Table 2. Effect poultry compost and foliar application of biostimulants on yield attribute, grain and straw yields (kg ha$^{-1}$) in finger millet**

| TREAT MENTS | Yield attributes | | | Grain and straw yield (kg ha$^{-1}$) | | Harvest Index |
|---|---|---|---|---|---|---|
| | Number of fingers ear head$^{-1}$ | Fingers length (cm) | Number of grains ear head$^{-1}$ | Grain yield (kg ha$^{-1}$) | Straw yield (kg ha$^{-1}$) | |
| **MAIN PLOTS** | | | | | | |
| **$M_1$** | 5.50 | 6.20 | 1092 | 2689 | 5467 | 32.88 |
| **$M_2$** | 6.51 | 7.43 | 1264 | 3098 | 6058 | 33.75 |
| **$M_3$** | 8.08 | 8.98 | 1480 | 3504 | 6597 | 34.68 |
| **S.Ed.** | 0.09 | 0.10 | 17 | 73 | 74 | 0.37 |
| **C.D (P=0.05)** | 0.26 | 0.29 | 48 | 158 | 212 | 0.96 |
| **SUB PLOTS** | | | | | | |
| **$S_1$** | 4.36 | 5.21 | 950 | 2389 | 4968 | 32.42 |
| **$S_2$** | 7.27 | 8.19 | 1375 | 3316 | 6347 | 34.26 |
| **$S_3$** | 6.87 | 7.63 | 1290 | 3090 | 6079 | 33.62 |
| **$S_4$** | 6.95 | 7.72 | 1307 | 3139 | 6134 | 33.78 |
| **$S_5$** | 8.05 | 8.93 | 1472 | 3567 | 6675 | 34.78 |
| **S.Ed.** | 0.06 | 0.07 | 12 | 37 | 56 | 0.23 |
| **C.D (P=0.05)** | 0.13 | 0.14 | 24 | 83 | 119 | 0.48 |

**Main plot treatments**: $M_1$- Enriched FYM @ 750 kg ha$^{-1}$,$M_2$- Enriched pressmud compost @ 750 kg ha$^{-1}$, $M_3$- Enriched Poultry manure compost @ 750 kg ha$^{-1}$

**Sub plot treatments**: $S_1$- Control (Water spray), $S_2$- Foliar spray of humic acid @ 0.3%, $S_3$-Foliar spray of panchagavya @ 3%, $S_4$- Foliar spray of vermiwash @ 5%, $S_5$-Foliar spray of seaweed extract (All the foliar sprays were given at 20,40 and 60 DAT).

**Table 3. Effect poultry manure compost and foliar application of biostimulants on net return (Rs.ha$^{-1}$) and BCR in finger millet**

| Treatments | Cost of cultivation | Gross return (Rs.ha$^{-1}$) | Net return (Rs.ha$^{-1}$) | BCR |
|---|---|---|---|---|
| **$M_1S_1$** | 33192 | 50233 | 17041 | 1.51 |
| **$M_1S_2$** | 37512 | 66862 | 29350 | 1.78 |
| **$M_1S_3$** | 39192 | 59801 | 20609 | 1.53 |
| **$M_1S_4$** | 38142 | 61534 | 23392 | 1.61 |
| **$M_1S_5$** | 37242 | 71222 | 33980 | 1.91 |
| **$M_2S_1$** | 33192 | 54543 | 21351 | 1.64 |
| **$M_2S_2$** | 37512 | 74764 | 37252 | 1.99 |
| **$M_2S_3$** | 39192 | 71620 | 32428 | 1.83 |
| **$M_2S_4$** | 38142 | 72092 | 33950 | 1.89 |
| **$M_2S_5$** | 37242 | 82493 | 45251 | 2.22 |
| **$M_3S_1$** | 33192 | 60509 | 27317 | 1.82 |
| **$M_3S_2$** | 37512 | 86328 | 48816 | 2.30 |
| **$M_3S_3$** | 39192 | 81458 | 42266 | 2.08 |
| **$M_3S_4$** | 38142 | 82558 | 44416 | 2.16 |
| **$M_3S_5$** | 37242 | 91019 | 53777 | 2.44 |

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**RESEARCH ARTICLE**

# Sustainable Agriculture: A Road to Global Food Security

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**ABSTRACT**

For decades, farmers and scientists have been trying to increase agricultural output in the traditional way. However, agricultural development is hindered by various aspects. Inefficient fertilizer application leads to a vicious cycle of increased fertilizer application and environmental pollution. Diseases caused by bacteria, fungi, pests and viruses reduce the grain quality and yield. Various abiotic stress environments produced by anthropogenic and natural conditions inhibit the growth of plants. With an ever-increasing global population, some crucial measures need to be taken in order to maintain agriculture for global food security. An innovative solution that is both highly efficient and low in pollution is required to address this agricultural conundrum. Sustainable agriculture is crucial in this situation. The term "sustainable agriculture" refers to a broad range of methods. Sustainable agriculture is farming that maximizes the use of non-renewable resources while preserving the environment and assisting in the expansion of natural resources. The present review paper is written with the motive to identify the various sustainable agricultural practices across the world to provide an efficient and easy way to understand sustainable agriculture methods and their application in current times.

**Keywords:** Sustainable, agriculture, organic farming, green synthesis

## INTRODUCTION

One of the biggest problems of the modern era is providing food for everyone on the planet in a safe and sustainable manner. According to the United Nations report, the population of the world will reach about 8.5 billion by 2030, and then 50% more food will be required to meet the population demand (Singh et al., 2019). Consequently, increasing agricultural output and agronomic practices are crucial for ensuring the world's food security. Sustainable

agriculture aims to preserve the planet's capacity to support future generations while producing resources required for the current human population. There are many negative aspects of modern agriculture, such as threats to human health, loss of agricultural diversity, devastation of ecosystems resulting in the loss of biodiversity, and several animal welfare issues. Sustainable agriculture tackles these problems and places an emphasis on "planetary health," which holds that human well-being is correlated with the stability of the earth. Promoting socio economic fairness, making a profit, and preserving the health of the ecology are among its core values. Sustainable agriculture actively works to preserve and promote biodiversity because it has been a major factor in the mass extinction of plant and animal species on Earth. Modern agricultural techniques, which were founded on the green revolution, clearly increased food productivity, but at the expense of depleting natural resources. The externalization of agriculture resulted in a significant reduction in both environmental resilience and soil fertility. It requires a distinct strategy that should encourage farmers to apply their traditional expertise in order to produce more grains with the least number of outside inputs. This strategy is called sustainable agriculture, and it is currently desperately needed. Various nanomaterials are being used in farming in recent years which not only are eco-friendly in nature but also offer a good amount of stress tolerance (Arora et al., 2022). The integration of historically adopted healthful practices with contemporary agricultural system development is the source of sustainable agriculture practices. Thus, resource conservation and climate change resistance are key components of sustainable agriculture operations. The use of nano-sensors to detect nutrient deficiencies in soil and new agricultural technologies are further going to help farmers shift towards sustainable agriculture (Jha et al., 2023). Moreover, it is plausible that a greater proportion of traditional inputs, resources, or expertise may lead to a socioeconomic balance across diverse cultural groups.

**Goals of sustainable agriculture**

Sustainable agriculture aims to improve environmental quality, maintain economic viability, and meet human requirements for food, feed, and fiber. This demonstrates how sustainable agriculture takes into account social, economic, and environmental concerns all at once. The expectations of present and future generations (the sustainability's time horizon and capacity to persist over time), as well as social, entrepreneurial, and individual demands from farms, are just a few of the numerous factors that need to be taken into account in addition to the sustainability characteristics listed above. Any agricultural system will be deemed more sustainable if it can accomplish more objectives. Sustainability is therefore more than just a goal that has to be accomplished. Therefore, moving toward greater sustainability entails locating a community that will best fulfill the many objectives as a whole. Its execution is challenging, ambitious, and hampered by a number of issues. In order to achieve widespread adoption of sustainable agriculture, we have to make an effort to eliminate or minimize these barriers and bridge the implementation gap. Some of the most widely used sustainable agricultural practices are discussed further in this review article.

**Organic farming**

The main prediction for organic farming is the discontinuation of synthetic inputs and their substitution with organic alternatives, such as the application of organic manures and natural plant protection techniques rather than synthetic pesticides and fertilizers. However, Bhattacharyya and Chakraborty (2005) refute this by stating due to the nutritional security we must find a middle ground between organic and conventional farming. But organic farming is more than just eliminating chemicals from the soil. It is an all-encompassing strategy for enhancing the plant's health and the underlying productivity of the soil, which leads to the enrichment of the surrounding ecosystem and is a prerequisite for sustainable agriculture. According to the InternationalFederation of Organic Agriculture Movements, “Organic agriculture is a production system that sustains the health of soils, ecosystems and people”. Organic farming also permits water reusability which furthermore helps in preventing water contamination (Parizad S and Bera S. 2023). It also involves the farming of trap crops which helps to increase the water retention rate leading to a decrease in water consumption for agriculture purposes (Hassanali et al. 2008). Different soil organisms and living organic matter can improve the soil's ability to store nutrients and decrease the likelihood that these nutrients will be transported to surface or ground waters. Eventually, the processes that agricultural fields use to store nutrients will safeguard the surrounding lakes, rivers, and streams' and lakes' ecological health (Sivaranjani and Rakshit 2019).

Instead of using inputs that have negative impacts, sustainable agriculture depends on biological processes, biodiversity, and cycles that are tailored to the local environment. The main goal of organic farming is to create a self-sufficient, environmentally and financially sustainable agricultural system that produces clean food while enhancing the surrounding biodiversity and all of its constituent parts. This system must be in harmony with nature. Organic farming has become a viable alternative agricultural system that not only solves sustainability and quality issues, but also guarantees a profitable means of subsistence for India's rural communities. The rigorous use of chemical pesticides and fertilizers calls into question the idea of sustainability in all of its forms. Both the ecosystem and the food chain are harmed by it. Organic farming steers clear of all methods that might harm the agricultural ecology. In addition to producing nutritious food, it creates an ecological balance that guards against insect issues and poor soil fertility. India is blessed with all natural and human factors essential for the development of organic farming. Hence, the Government is rapidly working on promoting organic farming as an effective way to promote sustainable agriculture.

**Nano fertilizers for sustainable agriculture**

In the twenty-first century, nanotechnology is a new field that is expanding rapidly. The effects of natural or human-caused climate change on global agricultural productivity are negligible. Chemical fertilizers are a vital component of modern agricultural systems, but their long-term and continuous use poses significant risks to soil fertility, the environment, and the nutritional dynamics of the rhizosphere microbiome. Nanotechnology is a relatively young and rapidly developing field in the twenty-first century. There is very little impact that climate change, whether natural or man-made, has on world agricultural productivity. Although chemical fertilizers are an essential part of contemporary agricultural systems, their long-term and ongoing usage poses serious hazards to the environment, soil fertility, and the rhizosphere microbiome's nutritional dynamics. The application of NBFs is in its infancy in agriculture, yet it has promising potential for transforming traditional farming techniques into smart agriculture, compared to any of the existing strategies (Sharma et al., 2023).

**Advances in molecular and omics technology for sustainable agriculture**

Increasing population, climate change and exhaustive agricultural practices either influenced nutrient inputs of soil or generated biological and physico-chemical deterioration of the soils and affected agricultural productivity and agro-ecosystems. Concerns about crop productivity and food security are so pressing that microbe-based farming techniques need to receive more consideration. Because microbes are found in soil, water, and the air, and because of their intimate relationship with plants, sustainable agricultural objectives can be achieved. In the last several decades, the hunger for environmentally friendly agriculture has led to a hunt for helpful bacteria in crop production, soil fertilization, disease management, and plant growth stimulation. Using integrated advanced biotechnology, the crop microbiome provides new avenues for managing harmful bacteria and harnessing beneficial microbes. The microbiome of crops aids in the uptake of nutrients, growth, resistance to phytopathogens, and tolerance to abiotic stressors such salt, heat, and drought.

We only have a partial understanding of how the host's genotype, climate, mineral mobilization, soil composition, nutrient availability, interactions between the nexus of microbes, and interactions with other external microbiomes influence the functionality of the crop microbiome, despite its emergent functionality as a complex component of plant fitness. The least studied aspects of this type of crop microbiome, both cultivated and uncultivated, include its structure, composition, dynamics, and functional role. Modern biotechnological techniques are effective means of gathering the data needed to explore the microbiome and gather information for creating resistant and high-yielding crop varieties. The theoretical ideas and the practical use of these cutting-edge instruments in agricultural microbiome research are now fundamentally bridged by this information. Current omics techniques are a powerful tool for modeling, mapping, tracking, and managing the microbiome of crops. Crop microbiome identification using system biology and reverse engineering can aid in the development of future bioformulations for disease control, reclaiming strained agro-ecosystems, and increased crop yield.

Nano-system techniques in conjunction with crop microbiome triggering chemicals can aid in the development of nano-biopesticides and nano-biofertilizers. Comparing this combination to the conventional bioinoculants, there are

many advantages. They improve the likelihood of crops establishing, increase the accessibility of nutrients in the soil, and activate a variety of defensive systems in plants under stress (Rai et al., 2022).

**Green synthesis of metal based nano particle**

The large-scale use of conventional pesticides and fertilizers has put tremendous pressure on agriculture and the environment. Due to their great performance, low cost, and environmental friendliness, nanoparticles (NPs) have gained attention in a number of industries recently, particularly sustainable agriculture. Conventional techniques for producing nanoparticles are environmentally damaging and energy-intensive. On the other hand, chemical synthesis and plant-based synthesis of metal-based NPs are comparable, with the exception that biological extracts are used in place of the chemical reducing agent. This results in the production of NPs that are more affordable, effective, safe, and low-polluting, while also significantly reducing the usage of conventional chemicals. In order to increase yields and quality, green synthesized metal nanoparticles, or GS-MNPs, are commonly employed in agriculture.

Nanotechnology manipulates and monitors substances at the nanoscale by using scientific expertise in physics, chemistry and biology (Rai et al., 2008). Since nanotechnology has advanced so much over the last several decades, it is anticipated that we will live in a "NPs world." (Adeel et al., 2021a; Adeel et al., 2021b; Singaravelan and Alwar, 2015). NPs have unique optical, electrical, magnetic, chemical and mechanical properties due to their small size (<100 nm) and large specific surface area (Dinesh et al., 2012; Puay et al., 2015; Saif et al., 2016). Nanotechnology may significantly increase the productivity and quality of agricultural products while reducing the harmful environmental effects of pesticides and fertilizers. This is especially important in view of the numerous issues confronting agriculture, including extreme environmental stress, climate change, and food security. In addition, nanotechnology can alleviate various abiotic stresses through stimulating physiological processes during plant growth and improving the quality of soil and agricultural products (Pereira et al., 2021). The unique properties of NPs make them indispensable weapons for solving agricultural problems (Adeel et al., 2020). The reduction process of green synthesis is approximately the same as chemical reduction, except that the chemical reagents are replaced by plant extracts (leaves, fruits, roots or flowers) (Hussain et al., 2016a). Metal ions can be reduced faster to form stable metal NPs by the green synthesis method with less pollution, simple operation and low energy consumption (Iravani, 2011). In addition, the waste produced during the synthesis of plants-based NPs, are non-toxic and more accessible to treat than chemical and physical methods (Hussain et al., 2016b). It is worth noting that the method of green synthesis of metal nanoparticles (GS-MNPs) can be used to recover some noble metal ions, such as gold (Au) and silver (Ag), which greatly improves the economic benefits (Wang et al., 2009). Therefore, GS-MNPs have more advantages over traditionally synthesized NPs.

**Sustainable agriculture using arbuscular mycorrhiza**

Increased salt content, drought, heavy metals, and other environmental pressures commonly affect worldwide agriculture, restricting plant growth and yield, degrading soil quality, and posing a serious threat to global food security. It is crucial to develop methods for mitigating environmental pressures and to impose limitations on the use of chemical fertilizers in agricultural areas. Eco-friendly practices must be maintained in order to shield agricultural areas from the negative effects of stress. Plants do not now possess the sophisticated metabolic machinery required to address this problem or cope with the pressures.

According to research, arbuscular mycorrhizal fungi (AMF) play a crucial role in improving plant nutrient absorption, heavy metal immobilization and translocation, and growth-promoting characteristics. As such, AMFs may be useful agents for plant development in a variety of stressful conditions. The productive symbiosis and the functional bond between the plant and AMF may strengthen the regulatory mechanism that protects against the main obstacle, specifically stress. The compatibility of AMF with hyperaccumulator plants has also been bolstered by theoretical reasons and investigations on gene regulation (Chauhan et al., 2022).

**Endophytic Archae for sustainable agriculture**

Although archaea have been around for more than 3.5 billion years, little is still known about them despite the fact that they were just recently found in the plant endosphere. Sustainable agriculture may benefit from the use of archaeal endophytes, especially in light of climate change and rising food demand brought on by population expansion. Recent developments in research techniques that do not rely on culture have shown a diversified abundance of archaea worldwide from the phylum *Euryarchaeota, Crenarchaeaota,* and *Thaumarchaeota*, which are linked to important crops including maize, rice, coffee, and olive. Recent discoveries about the microbiome of plants have identified genes in archaea that may be involved in several metabolic processes in plants, such as the synthesis of amino acids and the regulation of phytohormones (Chow et al., 2022).

**Obstacles to implementation**

The implementation of sustainable agriculture (in a larger context) is not restricted by a single, clear barrier or specific limitation. The restriction is rather the result of a number of interrelated, situation-specific problems. We make no claims to the completeness of our classification, but we may divide the difficulties into four categories: theoretical, methodological, personal, and practical. The problems that stem from the definitions, understanding, and concept of sustainable agriculture are known as theoretical hurdles. Challenges pertaining to methodology are associated with the evaluation and implementation of sustainable agriculture. The farmer, who determines the sustainability of agricultural practices, and their traits are the source of personal challenges. Practical barriers are those that restrict action and adaptation to a more sustainable condition, even while these obstacles also have an impact on the prerequisites needed for implementation (such as information, knowledge, and intention). Practical hurdles are different from the first three in that they are related to social issues or impediments.

**India's approach towards sustainable agriculture**

Agriculture is the backbone of the Indian economy as well as society because of its high share in employment generation and livelihood creation. About 43% of India's geographical areas are used for agricultural activities. The industry receives its food, feed, and raw materials from this sector. Recognizing these facts, the Government of India has taken various initiatives at national and regional levels for healthy growth of agriculture. Despite this agriculture's share in GDP has declined rapidly in the recent past. Indian farmers are suffering from multiple issues such as high input cost, low profitability, land degradation, depleting water table and risks related to climate change. The approach adopted by India focuses on key factors like local climatic conditions, regional physiographic, availability of water resources, accessible technology mainly revolves around developing climate resilient agriculture which is suitable to local climatic conditions reviving natural methods of farming such as organic farming, mixed farming, crop rotation and harnessing the potential of dry land area or rain fed area agriculture in India. Apart from this, the Government of India has emphasized more on sustainable development of irrigation facilities with water use efficiency through promotion of micro- irrigation techniques. In addition, the government encourages farmers to diversify their operations and take up new ones including goat farming, chicken raising, animal husbandry, beekeeping, and wood plantations. Financial assistance is given to farmers in mountainous areas, particularly in North-Eastern India and the Western Himalayan states, to enable them to engage in sustainable horticultural practices.

The schemes related to dairy farming, food processing and infrastructure development fund reduce the farmers" dependence on agriculture. Recently the government of India has come up with the resolve of doubling farmers" income by 2022 through a seven-point strategy. The strategy focuses on irrigation, quality seeds, post-harvest management, marketing, insurance and ancillary activities. This strategy has to be incorporated with the principles of sustainable agriculture which only can help in achieving the goals of environmental health, economic profitability and socio-economic equity. Climate smart agriculture, which includes employing renewable resources like solar and biofuels, nitrogen-smart nutrient management, organic farming, agroforestry, ICT-based agro-advisories, and so on, must be quickly adopted by India.

## CONCLUSION

A farming system known as "sustainable agriculture" aims to preserve the planet's capacity to support future generations while also producing the resources required for the world's current human population. A huge population all over the globe is facing immense food shortage, pollution and adulterated food. Sustainable agriculture proves to be the best way to deal with this condition. The Government of India has a well- defined array of schemes to meet almost all the needs and issues related to the development of sustainable agriculture. But the solution and success lie in the seamless implementation of these programs. Agriculture is a state subject in India which leads to politicization and fragmentation of actions and solutions related to it. On the national front there is a need to develop a consensus with the states for executing a national agenda on sustainable agriculture. Furthermore, by teaching and assisting farmers in adopting sustainable agricultural techniques, higher education institutions might support environmentally sustainable agriculture.

The progress in sustainable agriculture depends more on the development of organic farming. It's high time to take strategic and effective steps to overcome the constraints in the way of organic farming. Sustainable agriculture employs many of the natural living organisms like Archaeal endophytes and Arbuscular mycorrhiza for pollution free, safe and sustainable agriculture. Even in places with harsh weather circumstances like a drought, flood, etc., these may provide food security. In other areas of agriculture, such as plant growth promotion, plant disease, insect/pest management, fungicidal agent, food security for food packaging, extending the shelf life and preventing spoilage, and other uses, other techniques like nano fertilizers and green synthesized MNPs may be beneficial. Because microbes are found in soil, water, and the air, and because of their intimate relationship with plants, sustainable agricultural objectives can be achieved. In the last several decades, the hunger for environmentally friendly agriculture has led to a hunt for helpful bacteria in crop production, soil fertilization, disease management, and plant growth stimulation. Using integrated advanced biotechnology, the crop microbiome provides new avenues for managing harmful bacteria and harnessing beneficial microbes. The microbiome of crops aids in the uptake of nutrients, growth, resistance to phytopathogens, and tolerance to abiotic stressors such salt, heat, and drought. There should be intensive research about the plant microbiome and their application for sustainable agriculture.

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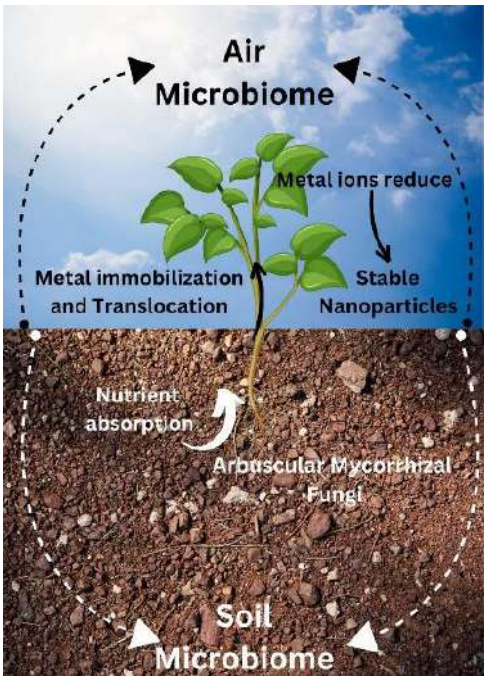


**Figure 1: Role of microbiome in sustainable agriculture**

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**REVIEW ARTICLE**

# A Systematic Review on Efficacy and Safety of N-Acetylcysteine and Carnitine in the Treatment of Hyperinsulinemia for Polycystic Ovary Syndrome

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## ABSTRACT

A systematic review addressing the efficacy and safety of N-Acetyl Cysteine (NAC) and Carnitine in the treatment of hyperinsulinemia for PCOS was done. The literature search was conducted from January 2015 to July 2022 using particular search terms in databases (Pubmed, Cochrane Library, and Scopus) and the literature was screened based on inclusion and exclusion criteria. A total of 118 records were selected initially. Nine articles were included, of which four were about NAC and five were about carnitine, based on the inclusion and exclusion criteria. The chosen studies included 924 patients in total. NAC and Carnitine significantly reduced BMI, fasting insulin, fasting blood sugar, Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), testosterone, sex hormone binding globulin (SHBG) and showed improvement in HOMA index and low-density lipoprotein (LDL). Carnitine significantly decreased insulin resistance (IR), Total cholesterol, and Triglycerides (TG) than NAC. NAC improved hormonal profile in hyperinsulinemic patients with PCOS. In women with PCOS, carnitine improves insulin resistance, hypoadiponectinemia, polycystic ovaries, and irregular menstruation.

**Keywords:** PCOS, hyperinsulinemia, N-acetyl cysteine, Carnitine

## INTRODUCTION

Polycystic ovarian syndrome or PCOS, is a metabolic as well as endocrine disorder that generally disturbs women of reproductive age [1-6].Followingthe Rotterdam criteria, PCOS is diagnosed by the existence ofthe following criteria including oligo-ovulation or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovary morphology (PCOM) on ultrasound[7-11].PCOS affects between 3.7 and 22.5 percent of people in India.Traditional cardiovascular risk factors including hypertension, type-2 diabetes mellitus, anddyslipidemiaas well as non-traditional cardiovascular risk factors such as mood disorders like anxiety and depression are more prevalent in PCOS patients. Approximately 30–60% of women with PCOS were overweight or obese. PCOS is a complex metabolic illness with a strong correlation to insulin resistance, which causes hyperinsulinemia. It is likely that both elevated insulin production and decreased insulin clearance lead to hyperinsulinemia. Additionally, 10% of individuals develop type 2 diabetes mellitus [12]. The coexistence of obesity and insulin resistance raises the likelihood of having type 2 diabetes mellitusand cardiovascular disease in PCOS patients at a young age. Medicines that improve insulin sensitivity have an important function in the treatment of PCOS [13-17].

Hyperandrogenism and IR worsen each other, hence insulin-sensitizing drugs enhance the clinical and biochemical features of PCOS. An antioxidant called N-acetylcysteine is produced from the amino acid L-cysteine.Thiol/sulfhydryl (R-SH/-SH) groups, which are antioxidants, are present in NAC [18]. It transforms into metabolites that improve detoxification, promote the synthesis of glutathione, and immediately break down free reactive oxygen species. It protects against hyperinsulinemia, hyperglycemia, and infertility.In PCOS patients, it reduced serum androgen levels, improved homocysteine levels, and decreased lipid profiles [19-22].

Carnitine is derived from the amino acid lysine and methionine. L-carnitine (LC), the most biologically active form, carries the fatty acids into the mitochondria, which are necessary for the generation of metabolic energy. [23,24]. The main acetyl ester of LC, acetyl-L-Carnitine (ALC), has been demonstrated to enhance reproductive functioning in PCOS by having antioxidative properties.The use of ALC in the treatment of IR has gained attention as it plays an important role in aggregating acyl-CoA derivatives that induce IR[16,25,26]. Carnitine is beneficial for weight loss, insulin function, glucose tolerance, and fatty acid metabolism[27-29]. NAC and Carnitine have beneficial effects in treating symptoms of PCOS. Hence, we planned to conduct a systematic review of the efficacy and safety of NAC and Carnitine on hyperinsulinemia in PCOS patients.

## MATERIALS AND METHODS

**Search Strategy and Study Selection**

This systematic review was performed in compliance with the "Preferred Reporting Items for Systematic Review and Meta-Analysis" (PRISMA) guidelines. The searched database includes PUBMED, Cochrane Library, and Scopus from January 2015 to July 2022. Search terms were PCOS, NAC, N- Acetyl Cysteine, Carnitine, and Hyperinsulinemia. Randomized controlled trials of NAC and Carnitineand articles that were published in English were included. The studies withnon-randomized controlled trials, no full text, duplication, case reports, brief reports, conference proceedings, review articles, observational cohort studies, and non-randomized controlled trials were excluded.

**Data extraction and analysis**

To compile the information and evaluate the literature, two authors worked independently. Two authors discussed and resolved discrepancies in the results summary. Reading the abstract and title served as the first screening while reading the entire content served as the second screening. PRISMA flow chart was used to define the selection of studies.

Two authors independently pull out the following information from each study which includes author name, number of subjects, year of study, inclusion criteria, exclusion criteria, study design, treatment, duration of treatment, outcomes of treatment, and adverse effects of NAC and Carnitine.

**Assessment of quality of included studies**

The quality of each study was evaluated by two authors independently using the Joanna Briggs Institute (JBI)checklist for randomized controlled trials. JBI comprises of 13 questionnaires. Each questionnaire was answered with yes, no, unclear, and not applicable. The overall response tothe questionnaire had three possible judgments: include, exclude, and seek further info.

## RESULT

A total of 118 studies were identified by the search strategy. The selection process of the study is shown in Figure 1 using the PRISMA flow diagram. 39 studies were excluded due to duplication,63 studies were excluded for not meeting the inclusion requirements and 9randomized controlled Clinical studies that complied with the inclusion criteria were selected. The Characteristics of selected studies of NAC and Carnitine were summarized in Tables 1 and 2 respectively. The result of the quality assessment using the JBI critical appraisal checklist for Randomized controlled trials were shown in Table 3

**N-acetylcysteine**

Aqrawi JG et al, confirmed that there was a substantial reduction in BMI, hirsutism, FSH, LH, FSH/LH ratio, testosterone level,and improved menstrual irregularity and follicle maturation in the NAC group. Kumar MA et al, mentioned that there was a significant reduction in body weight, fasting blood sugar (FBS), LH, FSH, LH/FSH ratio, and glucose/insulin ratio in the NAC group. Nemati Met alsuggested that after 8 and 12 weeks of treatment with NAC, a substantial decrease in mean BMI, total testosterone levels, hirsutism score, and fasting insulin level. Sex hormone binding globulin, FBS decreased only after 12 weeks of NAC treatment. Endometrial thickness increased after 12 weeks of treatment with NAC. Mature follicle number, LH/FSH ratio, LH, FSH, and E2 have no significant difference after long- and short-term treatment with NAC. Javanmanesh F et alindicated that NAC showed more improvement in BMI, FBS, fasting insulin, Homeostasis model assessment (HOMA) index, and LDL compared with metformin.

**Carnitine**

Tauqir S et al, confirmed that there was a substantial decrease in body weight, BMI, fasting serum insulin, the homeostasis model assessment for insulin resistance (HOMA-IR) index, fasting glucose, LH levels, and follicular volume of both ovaries and improvement in testosterone levels in combo group (Metformin + Pioglitazone + Acetyl-L-Carnitine) compared to the Metformin with Pioglitazone group. The serum FSH did not decrease in the groups. According to Ibrahim El Sharkwy et al., the carnitine group significantly reduced its levels of free testosterone, FSH, FG, FI, HOMA index, LDL cholesterol, total cholesterol levels, and triglycerides while also showing significant improvements in menstrual regularity, ovulation rate, pregnancy rate, FG/FI ratio, and HDL cholesterol. There was no apparent difference in BMI in both groups. Jamilian M et al, showed that there was a decrease in weight, BMI, FPG, insulin, HOMA-IR, insulin resistance, triglycerides, totalcholesterol,and LDL cholesterol and improved Quantitative insulin sensitivity check index (QUICKI) in the Carnitine group. Jamilian H et al, mentioned that there was a significant reduction in weight, and BMI in the Carnitine group compared to placebo. Samimi M et al, suggested that significant reduction in weight, WC, HC, FPG, serum insulin levels, HOMA-IR, and Dehydroepiandrosterone sulfate (DHEAS) in the Carnitine group compared to the placebo.

## DISCUSSION

Insulin resistance and Hyperinsulinemia are more common in women with PCOS. Insulin-sensitizing medications enhance the clinical and biochemical characteristics of PCOS because insulin resistance and hyperandrogenism aggravate each other [17,29]. NAC has been found to improve insulin resistance and hormonal levels. NAC and metformin improved lipid profiles, BMI, AUB, fasting blood sugar, and insulin, but NAC was more effective than metformin.Among individuals with CC-resistant PCOS, NAC and metformin therapy dramatically enhanced ovulation and pregnancy rates. When compared to patients receiving metformin alone, participants treated with NAC had somewhat greater rates of ovulation and pregnancy during the first and second cycles. Body mass index (BMI), menstrual regularity, and serum levels of various hormones were all reduced significantly in N-acetyl cysteine plus metformin than metformin alone.Metformin plus N-acetyl cysteine is preferable to metformin alone for the purpose of influencing follicle development, serum LH levels, & the menstrual cycle. Because of its lack of side effects, N-acetyl cysteine can be considered as an appropriate substitute for insulin-lowering PCOS treatment [32-33]. Carnitine supplementation resulted in a substantial reduction in body weight, BMI, WC, and HC in women with PCOS when compared to placebo. The combination of L-carnitine and metformin likely showed a significant improvement in lipid profile. Adding metformin with L-carnitine improved insulin resistance, ovulation rate, and pregnancy rate than metformin alone [17]. The group that took carnitine showed significant improvements in their fasting blood glucose levels, HOMA-IR, and serum insulin levels [29]. Carnitine supplementation resulted in a considerable reduction in FPG, insulin levels, DHEAS, and HOMA-IR but did not affect QUICKI. Similar findings reported that co-supplementation of chromium and Carnitine after treatment has significantly low FPG, HOMA-IR, and insulin resistance. Patients with PCOS are more likely to develop obesity due to hyperinsulinemia and androgen excess. PCOS Women received chromium and Carnitine co-supplementation for 12 weeks lost weight and had a lower BMI. L-Carnitine supplementation did not affect TG compared to placebo.When compared to a placebo, carnitine therapy significantly reduced plasma malondialdehyde (MDA) and increased plasma total antioxidant capacity (TAC). PCOS women who received Carnitine supplements for 12 weeks had substantial improvements in mental health parameters when compared to the placebo. When compared to a placebo, the weight and BMI changes were substantially reduced when taking supplements of carnitine [34-36]. Improvement was consistently seen in all anthropometric parameters, such as waist-to-hip ratio and BMI. Chromium and Carnitine co-administration improved lipid profiles except for HDL cholesterol levels in patients with PCOS [29,36].

## CONCLUSION

NAC improved hormonal profile in hyperinsulinemic patients with PCOS. NAC preserves more follicles in the ovary through its anti-apoptotic mechanism. In women with PCOS, carnitine improves insulin resistance, polycystic ovaries, irregular menstruation, and hypoadiponectinemia. It had a positive impact on weight loss along with the normalization of the menstrual cycle in women with PCOS. Consuming carnitine may help PCOS-affected women experience less oxidative stress as well as stress burden. Due to the lack of side effects, NAC and Carnitine may be a suitable alternative to insulin-lowering drugs for PCOS individuals.

**CONFLICTS OF INTEREST**

The author confirms no conflict of interest

**AUTHORS CONTRIBUTION**

Swetha S: Data Collection, original draft preparation
Janani S: Data Collection, original draft preparation
Sophia S: Data Collection
Jain Arvin Robert R: Data Collection
Mohamed Wasim Khan N: Data Collection
Jayasutha Jayram: Supervision, formal analysis, review, and editing of the manuscript

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**Table 1. Characteristics of selected studies of n-acetyl cysteine in PCOS:**

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Javanmanesh F et al [30] | 2015 | Randomized double-blind clinical trial | Total no. of patients = 94 (NAC group n=46 and Met Group n = 48) | Age between 20 and 36 years who were having PCOS according to the Rotterdam Criteria | Systemic, metabolic, and endocrine disorders, Cushing syndrome, congenital adrenal hyperplasia, hyperprolactinemia, thyroid disorders, and diabetes mellitus, history of peptic ulcer, and drug use for infertility | NAC Group 600 mg, three times a day. Met Group - 500 mg Met three times a day. | 24 weeks | NAC improved lipid profile and FBS and fasting blood insulin than Metformin. | GI discomfort in both groups |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Nemati M et al [31] | 2017 | Controlled clinical trial | Total no. of patients =108 (NAC group n=54 and Met group n=54) | Infertility for at least one year, having clomiphene resistance, patent tubes on hysterosalpingogra m, and normal semen analysis of the patients' spouses. | History of liver and kidney failure, diabetes mellitus, cardiovascular disease, Premature ovarian Failure, cushing's syndrome, any androgen-secreting tumours, chemotherapy and radiotherapy in pelvis cavity or surgery on ovaries | Received either Met (1500 mg/ day) or NAC (1800mg/day) with 100 mg/day of CC for 8 and 12 weeks. | 12 weeks | Administering NAC as an adjuvant for CC compared to metformin is recommended for improving hormonal profile and treating anovulatory infertility in hyperinsulinemia patients, especially women with PCOS resistant to CC. | Adverse effects were not determined. |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Kumar MA et al [32] | 2018 | A single-blinded randomized trial | Total no. of patients = 100 (Met Group n= 50 and NAC group n= 50) | Women of Age group between 18–37 and are diagnosed with PCOS. | Diabetes mellitus, hepatic or kidney diseases | Met Group - 500 mg thrice a day and NAC Group - 600 mg thrice a day for 12 weeks. | 12 weeks | NAC improved insulin resistance and hormone levels, and long-term health through improvement of peripheral insulin and reduction of side effects than Metformin. | A few participants experienced nausea, vomiting, diarrhoea and headaches. |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Aqrawi JG et al [33] | 2022 | Prospective, comparative clinical study. | Total no. of patients = 45 (Met group n= 19 and NAC + Met group n= 25) | Female 18- 45 years, oligo ovulation or anovulation, clinical or biochemical hyperandrogenism and/ or polycystic ovaries | Congenital adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, Cushing syndrome, androgen-secreting neoplasia, diabetes mellitus, medication changing insulin hemodynamic, medication affecting carbohydrate metabolism, hormonal drugs, multivitamins, | Met group - 500 mg three times daily.<br>NAC + Met group - 600 mg three times daily plus Met 500 mg three times daily | 3 months | NAC with Metformin is superior to Metformin alone, affecting the menstrual cycle, serum LH level, and follicle maturation. | Adverse effects were not determined. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | and anti-obesity drugs within last 3 months, severe concurrent cardiovascular disease, Severe hepatic or kidney disease | | | | |

CC- Clomiphene citrate; FBS- Fasting blood sugar; GI- Gastrointestinal; LH- Luteinizing hormone; Met- Metformin; NAC- N-Acetyl Cysteine

**Table 2. Characteristics of selected studies of carnitine in PCOS:**

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Samimi M et al [34] | 2016 | Randomized, double-blind, placebo-controlled trial | Total no. of patients=60 (Carnitine group n=30 and Placebo group n=30) | Age 18–40 years; oligo/ anovulation, hyperandrogenism, polycystic ovary morphology | Hyperprolactinaemia, Diabetes mellitus, thyroid disease, subjects following a special diet or consuming drugs with an effect on hormonal profile like oral contraceptives, ovulation induction agents and anti- obesity therapies in the last 3 months before enrolment. | PCOS patients received either 250 mg Carnitine supplements or a placebo. | 12 weeks | Carnitine group reduces weight, BMI, WC, HC and glycaemic control. It has no effect on lipid profile or free testosterone levels. | No side effects associated with the administration of Carnitine were observed. |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Jamilian H et al [35] | 2017 | Randomized, double-blind, placebo-controlled trial | Total no. of patients= 56 (Carnitine group n=28 and Placebo group n=28) | Age 18–40 years, oligo- and/or anovulation, biochemical signs of hyper-androgenism & polycystic ovaries | Pregnant women, individuals with metabolic diseases, thyroid disease, hyperprolactinemia, hypercortisolaemia, renal and liver diseases | 250 mg Carnitine supplements for 12 weeks and placebo. | 12 weeks | Carnitine improved mental health and oxidative stress indicators. | No side effects were observed in patients received Carnitine. |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| El Sharkwy I et al [16] | 2019 | A double-blinded randomized clinical trial | Total no. of patients = 274 (Carnitine group n=138 and Placebo group n=136) | Oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovary | Smokers, drug users, individuals with other reasons of infertility such as a male or tubal factor, endocrine abnormalities such as thyroid dysfunction & hyperprolactinemia | From 3-7 days of menstrual cycle, Group 1 = 150 mg/dCC plus L-Carnitine (3 g) & metformin 850 mg. After 1-week dose doubled as 1700 mg/dCC Group 2 = 150 mg/d of CC with metformin and placebo. | 15 months | Carnitine group enhances ovulation rate, pregnancy rate, menstruation regulation rate, insulin resistance & lipid profile. | The side effects were not determined. |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Jamilian M et al [36] | 2019 | Randomized, Double-Blind, Placebo-Controlled Trial | Total no. of patients= 54 (Carnitine group n= 27 and Placebo group n=27) | Overweight and obese women with PCOS diagnosed based on the Rotterdam criteria, and age 18–40 years. | Pregnancy, adrenal hyperplasia, androgen-secreting tumors, hyperprolactinemia, thyroid dysfunction, and diabetes. | Supplements (200g/ day chromium picolinate with 1000 mg/day Carnitine) or a placebo | 12 weeks | Carnitine supplements improved body weight, glycemic management and lipid profiles | Adverse effects were not determined. |

| AUTHOR NAME | YEAR | STUDY DESIGN | NO OF SUBJECTS (n) | INCLUSION CRITERIA | EXCLUSION CRITERIA | TREATMENT | DURATION OF TREATMENT | OUTCOMES OF TREATMENT | ADVERSE EFFECTS |
|---|---|---|---|---|---|---|---|---|---|
| Tauqir S et al [29] | 2021 | Double-Blind Randomized Clinical Trial | Total no. of patients =133 (Met+ pio group n=62 and Combo group n=71) | Oligo- or anovulation, hyper-androgenism, polycystic ovary morphology on ultrasound or increased ovarian volume | Pregnancy, hyper-prolactinemia, thyroid illness, cushing syndrome, late-onset congenital adrenal hyperplasia, on hormone replacement therapy | Combo group - metformin, pioglitazone, & ALC (500 mg, 15 mg, and 1500 mg). Met + Pio group - metformin + pioglitazone (500 mg, 15 mg) and placebo twice daily. | 12 weeks | Carnitine group improved insulin resistance, polycystic ovaries, menstrual irregularities & hypo-adiponectinemia | A few participants complained of GI issues after consuming carnitine. |

ALC- Acetyl L-Carnitine; BMI- Body mass index; CC- Clomiphene citrate; GI- Gastrointestinal; HC- Hip circumference; LDLR- Low density lipoprotein receptor; Pio- Pioglitazone; WC- Waist circumference

**Table 3. JBI critical appraisal checklist for Randomized controlled trials:**

| QUESTIONNAIRE | Aqrawi JG *et al* | Kumar M *et al* | Nemati M *et al* | Javanmaneshh F *et al* | Tauqir S *et al* | Jamilian M *et al* | El sharkwy I *et al* | Jamilian H *et al* | Samimi M *et al* |
|---|---|---|---|---|---|---|---|---|---|
| Was true randomization used for assignment of participants to treatment groups? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Was allocation to treatment group concealed? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Were treatment groups similar at the baseline? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Were participants blind to treatment assignment? | yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Were those delivering treatment blind to treatment assignment? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Were outcomes assessors blind to the treatment assignment? | Unclear | No | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear |
| Were treatment groups treated identically other than the intervention of interest? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Was follow up complete if not, were differences between groups in terms of their follow up adequately described and analyzed? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Were participants analyzed in the groups to which they were randomized? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Were outcomes measured in the same way for treatment groups? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Were outcomes measured in the reliable way? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Was appropriate statistical analysis used? | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Was trail design appropriate and any deviation from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trail | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

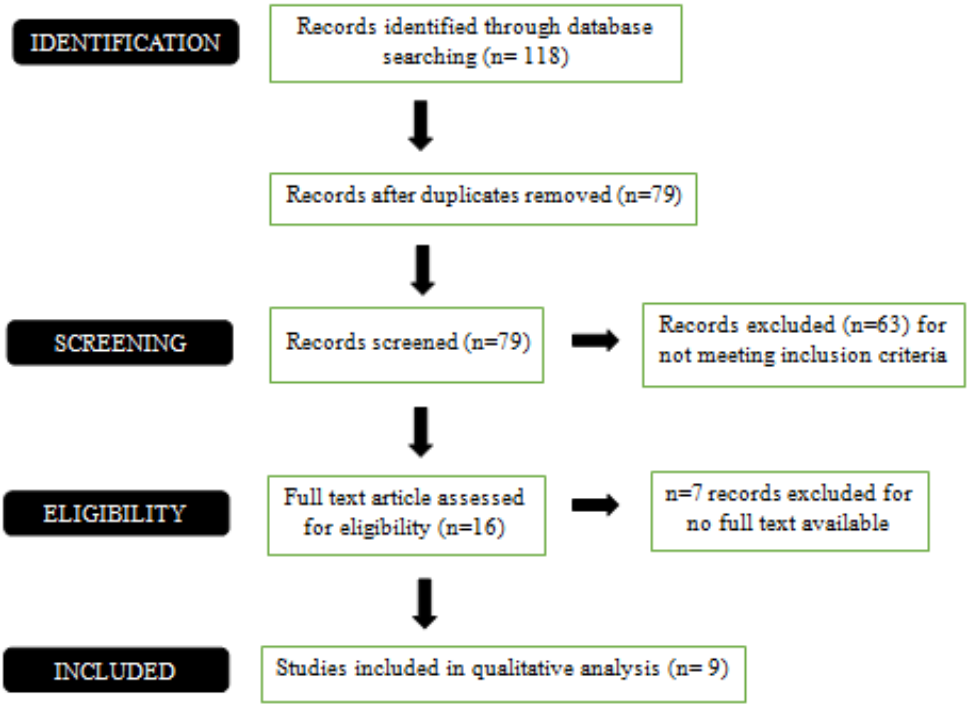


**Fig.1:PRISMA chart of the study selection process**

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**RESEARCH ARTICLE**

# GC - MS Profile of Ethanolic Root Extract of *Withania somnifera* (Ashwagandha)

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## ABSTRACT

In the present study phyto -constituents of root ethanolic extract were analyzed to check and identify the important phytochemicals by GCMS analysis. The present study indicates the presence of some essential pharmaceutical compounds- Benzo[b]thiophene-3-carboxylicacid, Hydrazine, N, N-dimethyl-N'-diethylboryl-, 7-acetoxy-2-acet, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, Propanamide,2-hydroxy-N-methyl-, 2-(Chloromethyl)-5-ethyl-1,3,4-oxadiazole, N-Nitroso-2,4,4-trimethyloxazolidine, Adenosine, N6-phenylacetic acid, ButylatedHydroxytoluene, . Benzo[b]thiophene-3-carboxylicacid,7-acetoxy-2-acet, Bonomycinhydrochloride, Propanamide,2-hydroxy-N-methyl-, 2-(Chloromethyl)-5-ethyl-1,3,4-oxadiazole, N-Nitroso-2,4,4-trimethyloxazolidine, 2-Amino-N-(4-fluorophenyl) benzamide, 2TBDMSderi, Tetradecane, and 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione. In the present investigation, 100 peaks were observed and identified. Out of 100 peaks, 61 peaks were found to be pharmaceutically important. Results indicate that the root of *Withania somnifera* contains many bioactive compounds that can be exploited to develop eco-friendly, plant-based nontoxic drugs to treat viral, bacterial, and fungal infections and also can treat cancer and some other diseases.

**Keywords**- GCMS analysis, *Withania somnifera,* Pharmaceutical compounds, Benzo[b]thiophene-3-carboxylicacid, -(Chloromethyl)-5-ethyl-1,3,4-oxadiazole.

## INTRODUCTION

*Withania somnifera* or Ashwagandha is an important Ayurvedic plant (1) belonging to the Solanaceae family(2). Ashwagandha plant grows up to 1.5 meters (3). Since ancient times *Withania somnifera* (Ashwagangandha) has been used to cure various diseases. Many Ayurvedic and Siddha drugs have been formulated by the use of whole plants or constituents of Ashwagandha. It is found in many countries like- India, Afghanistan, China, Nepal, Bhutan, and Bangladesh (4). Ashwagandha is widely cultivated in different parts of India- Rajasthan, Gujarat, Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Punjab, Haryana, Andhra Pradesh, Karnataka, and Tamil Nadu (5, 6). *Withaia somnifera* contains various secondary metabolites – Withanolides, flavonoids, terpenoids, tannins, saponins, phenolic compounds, and other useful compounds. Ashwagandha Roots manufactures Ayurvedic medicines, tonics, Chyavanprash, oral drops, haircare, and other healthcare products. In the Ayurvedic system of medicine, *Withania somnifera* roots are used as a Rejuvenating drug, tonic, Alternative pungent, astringent, Aphrodisiac, and Phthisis (7-9).

## MATERIALS AND METHODS

*Withania somnifera* plant samples including wild and developed verities were collected from various parts of India. Collected plant samples were verified by Dr. Prof. P Jayaraman PhD. Director- Plant anatomy Research center, Retd. Professor Presidency College, Chennai-5, Reg. No. of certificate- PARC/2021/4565. Each varieties were cultivated in Chennai field conditions

**Extract Preparation**

Plant root samples were washed with distilled water. Ethanol (95% v/v) was used to prepare the extract by the Soxhlet apparatus. The obtained plant root extract was further concentrated by a rotatory evaporator and then stored in the refrigerator at 4˚C for future analysis work.

**GCMS analysis**

*Withania somnifera* root extract was further diluted in ethanol and filtered through the Whatman filter paper number 41 to get particle-free extract dilution for GCMS analysis to check the active compounds present in the sample. Identification of active molecules was performed by a Gas Chromatography unit coupled with mass spectroscopy. A Shimadzu NX series coupled to Nexis GC -2030 Gas chromatograph was equipped with an AOC 20i automatic injector and a 30-meter, SH -I-.5SiI column (0.25mm ID, 0.25**µ**film thickness. The split injector was used for sample introduction and 10:1 was the split ratio. The oven temperature program was set to between 35˚C to 450˚C. The oven program was set to start at 35˚C for 2 minutes, followed by a steady increase up to 250˚C. then ramped to 450 The oven temperature program was programmed to begin at 35°C, hold for 2 minutes, then ramp at 20°C per minute to 450°C, and hold for 5 minutes. The helium Carrier gas was set to a 2 ml/minute flow rate (constant flow mode).

**Mass Spectrum**

A Direct connection with capillary column metal quadrupole mass filter pre-rod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanned from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2-second inter-scan delay. High-resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan.

**Mass spectrometry library search**

Identification of the components of the compound was compared to the known components stored in the mass spectra of NIST library V 11 provided by the instrument's software [10]. Also, GCMS metabolomics is used for similarity checking with the retention index [11].

## RESULTS AND DISCUSSION

In the present investigation -Benzo[b]thiophene-3-carboxylicacid, Hydrazine, N, N-dimethyl-N'-diethylboryl-, 7-acetoxy-2-acet, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, Propanamide,2-hydroxy-N-methyl-, 2-(Chloromethyl)-5-ethyl-1,3,4-oxadiazole, N-Nitroso-2,4,4-trimethyloxazolidine, Adenosine, N6-phenylacetic acid, ButylatedHydroxytoluene compounds indicates the presence of antioxidant and anti-cancer compounds. Benzo[b]thiophene-3-carboxylicacid,7-acetoxy-2-acet, Bonomycinhydrochloride, Propanamide,2-hydroxy-N-methyl-, 2-(Chloromethyl)-5-ethyl-1,3,4-oxadiazole, N-Nitroso-2,4,4-trimethyloxazolidine, 2-Amino-N-(4-fluorophenyl) benzamide, 2TBDMSderi, Tetradecane, 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione, compounds are known for antimicrobial and anti fungal activity. some important compounds like - Aceticacid,butylester, 2-Pentanone,4-hydroxy-4-methyl-, Ethylbenzene, p-Xylene, Dihydroxyacetone, Aminocyanoaceticacid, 1,2,3,4-Butanetetrol,[S-(R*, R*)]- Undecane, O-Methylisourea, Allene, Cyclohexasiloxane,dodecamethyl-, Tridecane, Acetamide, N, N', N''-methylidynetris-, 4-Heptafluorobutyroxypentadecane, Cyclohexasiloxane, dodecamethyl-, 1,3-Adamantanediacetamide, Cyclopentasiloxane, decamethyl-, Cycloheptasiloxane,tetradecamethyl-, Cyclohexanol,1R-4-trans-acetamido-2,3-trans-epoxy-, Oxacycloheptadec-8-en-2-one,(8Z)-, Hexatriacontane, Eicosane are industrially essential.

## Discussion

Phytoanalysis of Plant extract has been performed to check the presence of various active compounds in the sample. the present investigation revealed that *Withania somnifera* possesses many active compounds useful for Pharmaceutical, industrial, and healthcare purposes. It also indicates that the active compounds can be used for novel medication formulations.

**Conflict of Interest**- No

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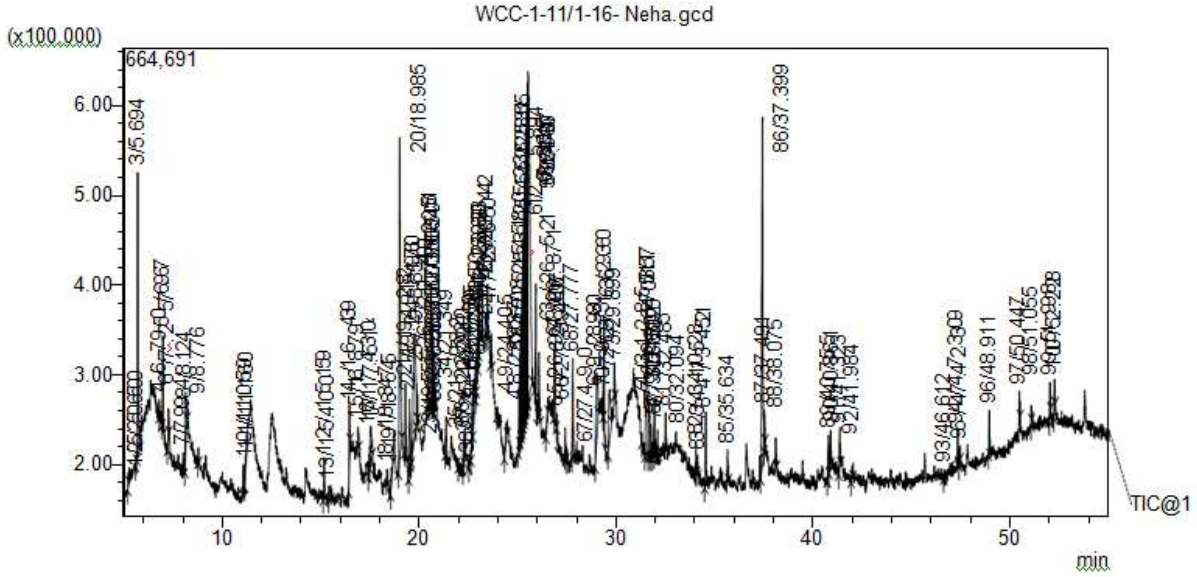
| Peak# | Ret.Time | m/z | Area | CompoundName | |
|---|---|---|---|---|---|
| 1 | 5.205 | TIC | 44882 | Benzo[b]thiophene-3-carboxylicacid,7-acetoxy-2-acet | anti-cancer, Anti-microbial, anti-oxidant, anti-inflammatory, anti-diabetic, anti-convulsant, and anti-tubercular activity (12) |
| 2 | 5.600 | TIC | 29055 | Hydrazine, N, N-dimethyl-N'-diethylboryl- | Medicine and cancer research (13) |
| 3 | 5.694 | TIC | 803723 | Acetic acid,butyl ester | Oils, perfume, nail polish remover, lacquers, and resins (14) |
| 4 | 6.790 | TIC | 71116 | 2-Pentanone,4-hydroxy-4-methyl- | Synthetic and natural resins and nitrocellulose production (15) |
| 5 | 6.967 | TIC | 291237 | Ethylbenzene | Styrene and polymer production (16) |
| 6 | 7.257 | TIC | 84068 | p-Xylene | Used as a solvent in rubber, leather, and printing industries (17) |
| 7 | 7.994 | TIC | 38238 | Benzene,1,3-dimethyl- | - |
| 8 | 8.124 | TIC | 314711 | Dihydroxyacetone | Artificial sweetener, FDA-approved agent for sunless tanning (18) |
| 9 | 8.776 | TIC | 28935 | Aminocyanoaceticacid | Used in pyrimidine synthesis (19) |
| 10 | 11.059 | TIC | 64012 | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | Antioxidant compound (20) |
| 11 | 11.160 | TIC | 108808 | 1,2,3,4-Butanetetrol,[S-(R*,R*)]- | diastereomer of erythritol (21) |
| 12 | 15.159 | TIC | 113231 | Undecane | Useful in the Flavor and fragrance industry (22) |
| 13 | 15.400 | TIC | 55807 | N-Acetyl-D-glucosamine | Influences cell signaling (23) |
| 14 | 16.439 | TIC | 173129 | 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- | Antioxidant activity (24) |
| 15 | 16.879 | TIC | 42596 | O-Methylisourea | Used for the production of antineoplastic compound fluorouracil and anthelmintic compound imidazole (25) |
| 16 | 17.430 | TIC | 83520 | Allene | For the synthesis of various chiral compounds. (26) |
| 17 | 17.514 | TIC | 228326 | Cyclohexasiloxane,dodecamethyl- | Used in hair and skin products, antiperspirants, and silicon polymer manufacturing (27) |
| 18 | 18.347 | TIC | 26056 | 2-Methyl-1-ethyl pyrrolidine | - |
| 19 | 18.545 | TIC | 54162 | Deferoxamine | To treat acute iron poisoning. To remove aluminum toxicity and to remove excess iron from anemia and thalassemia patients patients who have many blood transfusions. (28) |
| 20 | 18.985 | TIC | 1580861 | 5-Hydroxymethylfurfural | Inhibits sickle cell production in blood (29) |

| | | | | | |
|---|---|---|---|---|---|
| 21 | 19.282 | TIC | 234472 | N-(1,1-Dimethyl-3-oxobutyl)-2-methyl azetidine | - |
| 22 | 19.491 | TIC | 306162 | 1,4-Dioxane-2,3-diol,diacetate | Used in the synthesis of DNA duplexes and glyoxal for the synthesis of acetonides (30) |
| 23 | 19.555 | TIC | 28634 | 2,2'-(1,4-Piperazinediyl)bis[N-(m-tolyl)succinimide] | - |
| 24 | 19.760 | TIC | 951782 | Tridecane | Used in jet fuel research, manufacturing of paraffin products, and the rubber industry. (31) |
| 25 | 19.885 | TIC | 116038 | Bis(ethyl4-acetyl-5-oxohexanoato)copper(ii) | Used as an intermediate for the synthesis of maleate ( maleate is an important drug to treat allergies, itching nose/throat, and red eyes) (32) |
| 26 | 20.400 | TIC | 120112 | cis-2,3-Epoxyoctane | - |
| 27 | 20.561 | TIC | 713998 | Acetamide, N, N', N''-methylidynetris- | Used as a plasticizer and industrial solvent. (33) |
| 28 | 20.640 | TIC | 301225 | N-Heptyl-N'-(6-(6-[2-(heptyl-methyl-carbamoyl)-acety | - |
| 29 | 20.665 | TIC | 112190 | Bonomycinhydrochloride | Anti-bacterial compound (34) |
| 30 | 20.700 | TIC | 148187 | Melezitose | Attracts ants and food for bees. (35) |
| 31 | 20.730 | TIC | 68782 | Propanamide,2-hydroxy-N-methyl- | Chymotrypsin inhibitory and antimicrobial activity (36), Biomedical imaging for prostate cancer. (37) |
| 32 | 20.760 | TIC | 44284 | 4-Heptafluorobutyroxypentadecane | Used as a reference for Gas chromatography (38) |
| 33 | 20.820 | TIC | 42633 | .beta.-D-Lyxofuranoside,5-O-(.beta.-D-lyxofuranosyl)- | - |
| 34 | 21.349 | TIC | 146994 | Cyclohexasiloxane,dodecamethyl- | Cosmetics and personal care products. (39) |
| 35 | 21.982 | TIC | 41959 | .beta.-[5-Methyl-2-tetrahydrofuranyl]alanine | Increases muscle content (40) |
| 36 | 22.230 | TIC | 35047 | 7-Hydroxytomatidine, O, O, N-triacetate | - |
| 37 | 22.292 | TIC | 118391 | 1,2,4-Trioxolane,3,5-dipropyl- | Antimalarial activity (41) |
| 38 | 22.510 | TIC | 32596 | D-erythro-Pentose,2-deoxy- | It is a Metabolite (42) |
| 39 | 22.660 | TIC | 192063 | 8-Azabicyclo[3.2.1]octan-3-ol,8-methyl-,endo- | - |
| 40 | 22.705 | TIC | 312791 | 1,3-Adamantanediacetamide | Used in *etching masks (43)* |
| 41 | 22.845 | TIC | 55695 | 5-Methyl-5-octen-1-ol | - |
| 42 | 22.890 | TIC | 182921 | 3.beta.-Acetoxy-bisnor-5-cholenamide | - |
| 43 | 22.993 | TIC | 191055 | 3-Octenoicacid,decyl ester | - |

| | | | | | |
|---|---|---|---|---|---|
| 44 | 23.020 | TIC | 162750 | N-Cyclopropyl-11-(3-hydroxy-5-pentyl phenoxy)undec | - |
| 45 | 23.360 | TIC | 89618 | 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsil | - |
| 46 | 23.442 | TIC | 174293 | 4-(3-Aminophenoxy)phthalic acid,3TMSderivative | - |
| 47 | 23.650 | TIC | 87626 | 3,3,5-Triethoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsil | - |
| 48 | 23.937 | TIC | 49518 | | - |
| 49 | 24.405 | TIC | 116802 | Arachidamide,N-hept-2-yl- | Metabolite (44) |
| 50 | 25.085 | TIC | 352999 | 2-Formyl-9-[.beta.-d-ribofuranosyl]hypoxanthine | - |
| 51 | 25.145 | TIC | 430444 | 1,8-Nonadien-3-ol | - |
| 52 | 25.180 | TIC | 364802 | Dimethylmalonicacid,cis-4-methylcyclohexylpentade | Fatty acid synthesis inhibitor (45) |
| 53 | 25.235 | TIC | 616261 | Pentatriacontane,1-bromo- | - |
| 54 | 25.285 | TIC | 685218 | 6-Amino-1-.beta.-d-ribofuranosylimidazo[4,5-c]pyridi | - |
| 55 | 25.315 | TIC | 616323 | 2-(Chloromethyl)-5-ethyl-1,3,4-oxadiazole | Anti-microbial and anti-oxidant activity (46) |
| 56 | 25.385 | TIC | 1082439 | N-Nitroso-2,4,4-trimethyloxazolidine | Anti-microbial and anti-inflammatory. (47) |
| 57 | 25.415 | TIC | 583283 | Acetic acid,chloro-,2-butoxy ethyl ester | - |
| 58 | 25.440 | TIC | 810381 | Adenosine, N6-phenylacetic acid | Anti-inflammatory properties. (48) |
| 59 | 25.480 | TIC | 533207 | 4-CHLOROBUT-2-EN-1-OL | - |
| 60 | 25.507 | TIC | 2594849 | 2-(Isobutoxymethyl)oxirane | - |
| 61 | 25.894 | TIC | 334806 | Cyclopentasiloxane,decamethyl- | Hair spray, deodorants, sunblock, and other skincare products. (49) |
| 62 | 26.067 | TIC | 178682 | Cycloheptasiloxane,tetradecamethyl- | Skin conditioning agent, anti-caking agent (50) |
| 63 | 26.521 | TIC | 72370 | Hexanamide,3,5,5-trimethyl-N-hept-2-yl- | - |
| 64 | 26.871 | TIC | 304037 | ButylatedHydroxytoluene | Anti-oxidant, animal feed is also used as a preservative in food. (51) |
| 65 | 26.979 | TIC | 45705 | Pregnan-20-one,3,17,21-tris[(trimethylsilyl)oxy]-,O-( | Also reported in f *Mentha arvensis* oil (52) |
| 66 | 27.394 | TIC | 86225 | 2-Amino-N-(4-fluorophenyl)benzamide,2TBDMSderi | Anti-microbial agent (53) |
| 67 | 27.490 | TIC | 34987 | Dodecanedioic acid,2TBDMS derivative | Human metabolite, alcohol dehydrogenase inhibitor (54) |
| 68 | 27.777 | TIC | 325063 | 2-Amino-N-(4-fluorophenyl)benzamide,2TBDMSderi | Anti-microbial agent (55) |
| 69 | 28.980 | TIC | 476576 | 3-Isovaleryloxytropane | Used for the treatment of overactive bladder. (56) |

| | | | | | |
|---|---|---|---|---|---|
| 70 | 29.295 | TIC | 32276 | Octahydro-1H-cyclopenta[b]pyridin-4-ylacetate | - |
| 71 | 29.360 | TIC | 372198 | Tetradecane | Anti-bacterial and anti-fungal activity (57) |
| 72 | 29.636 | TIC | 417289 | Cyclohexanol,1R-4-trans-acetamido-2,3-trans-epoxy- | Used in the manufacturing of Insecticide, and soap (58) |
| 73 | 29.899 | TIC | 70415 | 1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(trimethyl siloxy)h | - |
| 74 | 31.285 | TIC | 47436 | 3,6,9,12-Tetraoxa-15-azapentacosan-1-ol,TMSderivat | - |
| 75 | 31.517 | TIC | 1085692 | Tropigline | Alkaloids in Withania somnifera can bind with SARS CoV -2 and human-targeted proteins. (59) |
| 76 | 31.640 | TIC | 286652 | Succinicacid,non-4-enyltetradecyl ester | Used as a neutraceutical, radiation protective, and anti-ulcer drug (60) |
| 77 | 31.760 | TIC | 122046 | Octane,2,3,6,7-tetramethyl- | - |
| 78 | 31.885 | TIC | 91518 | 6-Aza-2-thiothymine | Pharmaceutical intermediate (61) |
| 79 | 31.920 | TIC | 155262 | 2-Hydroxymethyl-2,6,8,8-tetramethyltricyclo[5.2.2.0(1 | Reported in root ethanolic extract of *Premna serratifolia.* (62) |
| 80 | 32.094 | TIC | 58998 | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | Possess high corneal permeability with strong and stable interactions with fungal virulence cellobiose dehydrogenase, (63) |
| 81 | 32.485 | TIC | 106875 | (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol | - |
| 82 | 34.028 | TIC | 74275 | Heptadecane | Plant metabolite. (64) |
| 83 | 34.115 | TIC | 41900 | Benzene,1,1'-[1,2-ethanediylbis(oxy)]bis- | - |
| 84 | 34.521 | TIC | 256182 | Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl | - |
| 85 | 35.634 | TIC | 92830 | 2'-Methylene-1-tosyl-1'-((trifluoromethyl)sulfonyl)spiro | - |
| 86 | 37.399 | TIC | 1532001 | n-Hexadecanoicacid | Anti-inflammatory agent (65) |
| 87 | 37.491 | TIC | 112575 | 6-Ethyl-4,5,7-trithia-2,8-decadiene | - |
| 88 | 38.075 | TIC | 46212 | Hexadecanoic acid,2-methyl-, methyl ester | It Can be used to treat *hepatic dysfunction. (66)* |
| 89 | 40.755 | TIC | 156871 | Oxacycloheptadec-8-en-2-one,(8Z)- | Used as a perfume base (67) |
| 90 | 40.861 | TIC | 131741 | Chloromethyl4-chlorododecanoate | - |
| 91 | 41.323 | TIC | 150029 | 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | Antifungal activity( 68) |
| 92 | 41.984 | TIC | 53246 | 1-Methyl-3-propan-2-ylurea | - |
| 93 | 46.612 | TIC | 32478 | 6H-1,2,5-Oxadiazolo[3,4-E]indole-6,8a-diol,4,5,5a,7, | - |
| 94 | 47.309 | TIC | 75966 | Hexatriacontane | Used as paraffin wax and in candle making (69) |
| 95 | 47.423 | TIC | 42679 | Hexadecanoic acid,1-(1-methyl | It exhibits *antioxidant,* |

| | | | | | |
|---|---|---|---|---|---|
| | | | | ethyl)-1,2-ethanediyle | *hypocholesterolemic, and antiandrogenic properties. (70)* |
| 96 | 48.911 | TIC | 138466 | Eicosane | Use to make candles and paraffin wax. (71) |
| 97 | 50.447 | TIC | 75797 | Tetratriacontane | Plant metabolite (72) |
| 98 | 51.055 | TIC | 23933 | 2,2'-(1,3-Phenylene)bis[4-(2-thienylmethylene)-5(4H)- | - |
| 99 | 51.998 | TIC | 105966 | Eicosane | Use to make candles and paraffin wax. (71) |
| 100 | 52.228 | TIC | 97686 | 5,9,13,17-Tetramethyl4,8,12,16-octadecatetraenoicaci | - |



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**RESEARCH ARTICLE**

# Performance Enhancement of Grid-Integrated PV System with Fuzzy Plus Fractional Order Notch Filter

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## ABSTRACT

This paper presents the power quality improvement of the three-phase grid-integratedPV system with a fractional notch filter and fuzzy control scheme. The interconnection of the unbalanced load develops the distortions of the grid/load current. The harmonics in grid current is compensated and enhance the transient performance of the system by employing fractional notch filter and fuzzy controllers. The Fractional notch filter (FONF) can estimate the active components from the distorted load current. Fuzzy controllers are positioned at the PV side to improve the performance of the DC link capacitor. The combination of both FONF and fuzzy controllers operates together to develop pulses for the voltage source converter (VSC). An efficient operation of the VSC compensates the harmonic current and makes stable the grid/load currents. To demonstrate the proposed method's effectiveness, a simulation study was performed for different scenarios. For steady-state balanced loads, dynamic loads, and input PV source variations also obtained transient free grid current by utilizing the proposed control approach.

**Keywords:** PV Inverters, Voltage stability, Fuzzy Logic Controller

# INTRODUCTION

Renewable energy sources (RES) have emerged as a cornerstone of modern energy systems, offering a transformative solution to the challenges of climate change, energy security, and sustainable development. Unlike conventional fossil fuels that deplete over time and contribute to environmental degradation, renewable energy sources harness the power of nature's abundant resources such as sunlight, wind, water, and geothermal heat to generate clean, inexhaustible energy.PV power production increasing rapidly due to relatively low operating and maintenance costs compared to other forms of power generation [1-2]. PV has no moving parts and generally requires only occasional cleaning and basic maintenance. Photovoltaic (PV) systems power generation shares much share in RES sources [3]. For reliability operation, photovoltaic (PV) systems are integrated into the grid. Grid-connected PV systems provide reliable power over the Islanded PV power generations. One of the primary benefits of grid-connected PV systems is the potential for significant cost savings on electricity bills [4].

Grid-connected PV systems can experience power quality issues, especially when connected to non-linear loads. Non-linear loads are devices that do not draw a sinusoidal current waveform from the grid, causing distortions and imbalances in the voltage and current waveforms [5]. Non-linear loads, such as computers, variable speed drives, and electronic devices, can introduce harmonic currents into the system. These harmonic currents can lead to distorted voltage waveforms, causing equipment malfunction, overheating, and reduced efficiency. The harmonics can also interfere with other sensitive equipment connected to the same grid.Rapid changes in the PV system's output due to cloud cover or other factors can cause voltage fluctuations in the grid. Non-linear loads can exacerbate these fluctuations, leading to voltage sags, swells, or flickering lights, which can impact the operation of sensitive equipment. Non-linear loads can lead to unbalanced currents in the three phases of a three-phase grid system [6]. This unbalance can result in unequal voltage levels across phases, potentially causing equipment damage, overheating, and inefficiencies. Non-linear loads can create sudden spikes in current demand when they switch on or off. These transients can lead to voltage drops in the grid, affecting the performance of both the PV system and connected loads {7-9]. In this paper, a fractional order notch filter is utilized to estimate the active power components from the distorted load current instead of abc/dqo park transformation theory [10]. The proposed FONF-based VSC enhancesthe overall performance of the grid-connected PV system.

**GRID-CONNECTED PV SYSTEM CONFIGURATION**

Fig.1 represents the three-phasegrid-tie PV system for a balanced load. Solar output voltage (D.C) is stored in a D.C capacitor. To extract maximum power from the incremental conductance MPPT technique [11-13] is adopted. Bi-polar VSC is used to connect the PV system to the grid. The RC filters can eliminate the higher frequency components generation from converter switches. To reach the power quality issues VSC operating pulses are generated with FONF and fuzzy rules

**CONTROL SCHEME**

A fractional-order notch filter (FONF) is a type of filter that is designed to attenuate or reject specific narrow ranges of frequencies while utilizing fractional-order calculus concepts. FONF can offer more precise control over frequency responses compared to traditional integer-order filters. This precision is valuable for targeting specific harmonics generated by non-linear loads in the grid-connected PV system. By selecting appropriate fractional orders and center frequencies, the filter can effectively attenuate or eliminate these harmonics, thereby improving power quality. FONF is used to extract the active power from the distorted components of the load current when a grid-tied PV system is connected to unbalanced loads.

The MATLAB circuit diagram of proposed FONF and fuzzy control scheme to generate the pulses to operate the VSC is depicted in Fig.2b

**SIMULATION RESULTS**

To check the effectiveness of the proposed FONF and fuzzy control scheme grid-tied PV system performance is inspected under different conditions.

**Case 1: Performance of the system under constant load condition**

In this case, a load of 205W is connected to the load of the grid-interfaced PV system. The obtained simulation responses in this scenario such are voltage at PCC, grid currents, load current, and converter current are depicted in Fig3a- fig 3d respectively. PV produced (see fig.3g) 1.51kW, in this load utilizing 205W remaining surplus power surplus power 1.31kW delivered to the load. Fig.3e and Fig.3f illustrate the grid power and load power. From the THD plot (depicted in Fig.3h) the harmonic content in the grid current under this steady-state load condition is 3.76. It reveals that the proposed method compensates harmonic current adequately and provides better power quality.

**Case 2: Performance of the system under dynamic load condition**

In this condition, the performance of the system under dynamic behavior of load condition is observed. In this case load from the C phase is disconnected abruptly. This type of unbalanced load distorted the grid currents heavily. Fig. 4 (a) represents the simulation outputs of the line voltage (Vsab) and Converter currents (Iia, Iib, and Iic) and Fig. 4(b) shows the simulation outputs of the DC link voltage (Vdc), C- Phase Grid, load and converter currents (Igc, ILc, and Iic). As can be seen from Fig.4b grid currents ensure that the proposed FONF and fuzzy control scheme well compensate for the current harmonics and balance the grid-tied PV system. For unbalanced load conditions also stable grid current is obtained with the help of the proposed control scheme

## CONCLUSION

The power quality enhancement of the three-phasegrid-connected PV system with fractional notch filter and fuzzy control scheme design is reported in this paper. The Fractional notch filter (FONF) can estimate the active components from the distorted load current due to unbalanced loading effects. Fuzzy controllers are positioned at the PV side to improve the performance of the DC link capacitor. Both FONF and fuzzy controllers are responsible for producing pulses to operate the VSC. Simulation study performed for different conditions such as steady state balanced loads, dynamic loads, and input PV source variations. Obtained simulation responses it is clear the proposed FONF and fuzzy controllers operated the VSC efficiently to improve the quality of the grid/load current. The proposed control scheme solves the power quality problems that are harmonics distortion, reactive power burden on the system, and unbalancing of connected loads.

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**Table.1: Fuzzy rules**

| | Error (E) | | | | | |
|---|---|---|---|---|---|---|
| Change in error (ΔE) | | NegativeBig | NegativeSmall | Zero | PostiveSmall | PB |
| | NegativeBig | NegativeBig | NegativeBig | NS | NSm | Zero |
| | NegativeSma | NegativeBig | NSm | NS | Zero | PSm |
| | Zero | NegativeSm | NSm | Zero | PSm | PSm |
| | PostiveSmall | NegativeSm | Zero | PSm | PSm | PBig |
| | PostiveBig | Zero | PostiveSmall | PSm | PBig | PBig |

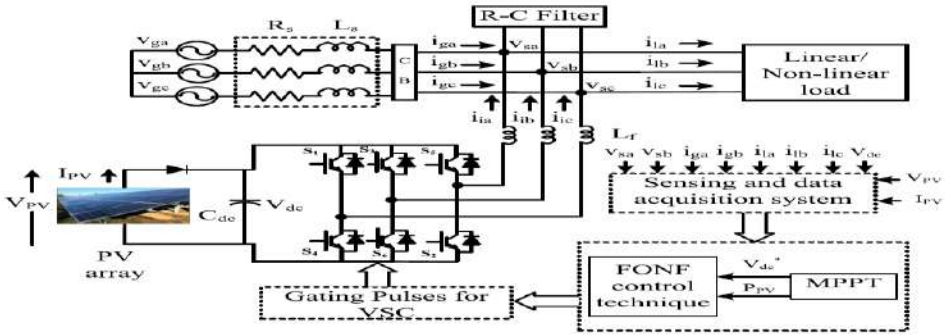


**Fig.1 Structure of the grid-connected PV system**

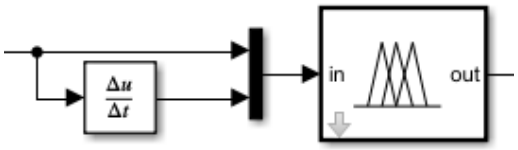


**Fig.2a. Structure of FLC controller**

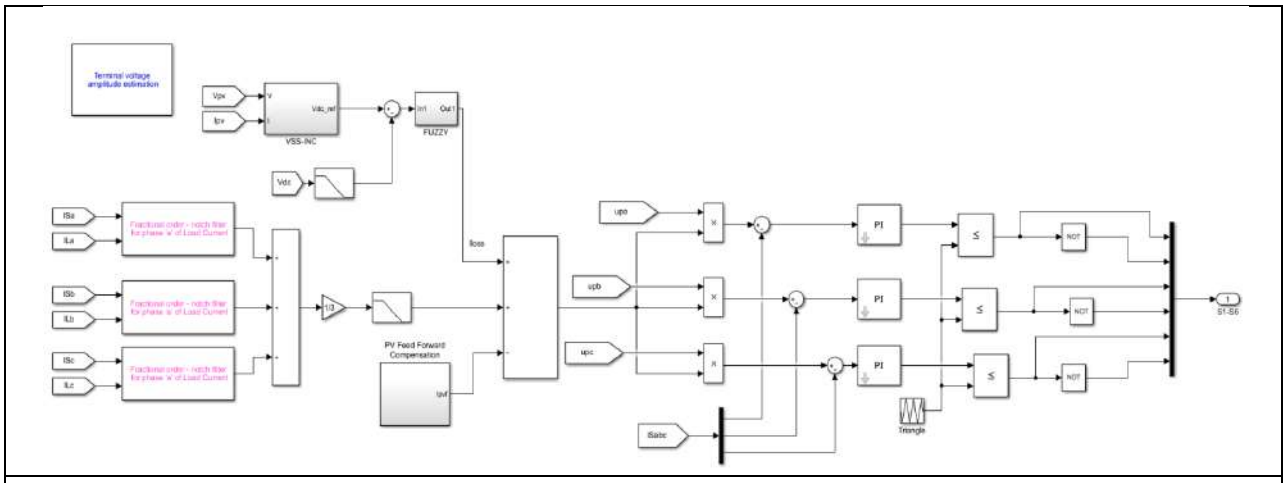


**Fig.2b Proposed Control Scheme**

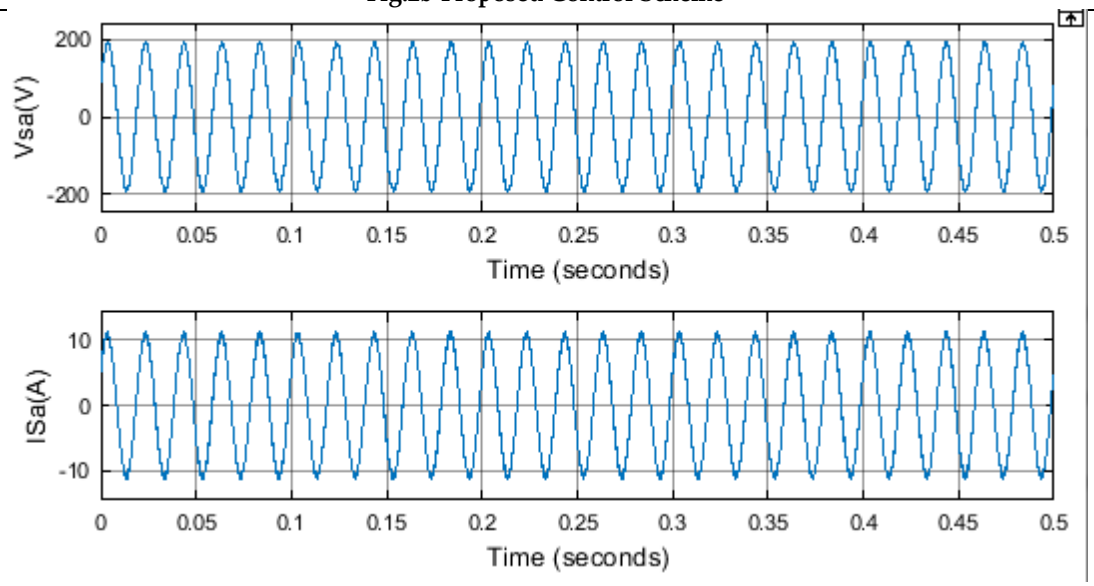


**Fig.3a Voltage at PCC ($V_{sa}$) and Fig.3b Grid currents ($I_{sa}$)**

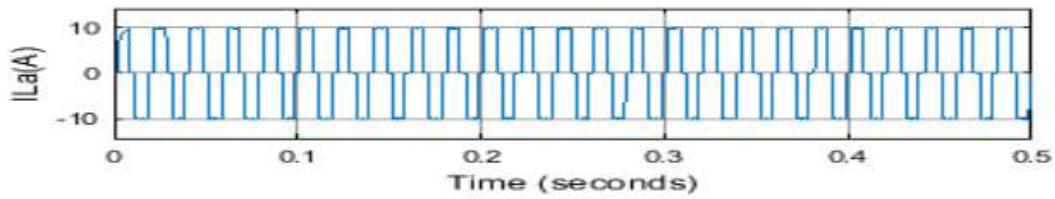


**Fig.3c load currents ($I_{La}$)**

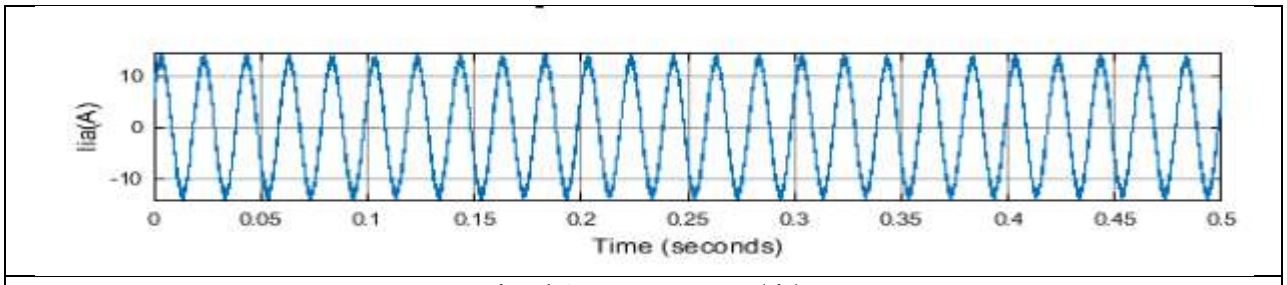


**Fig.3d Converter current (Iia)**

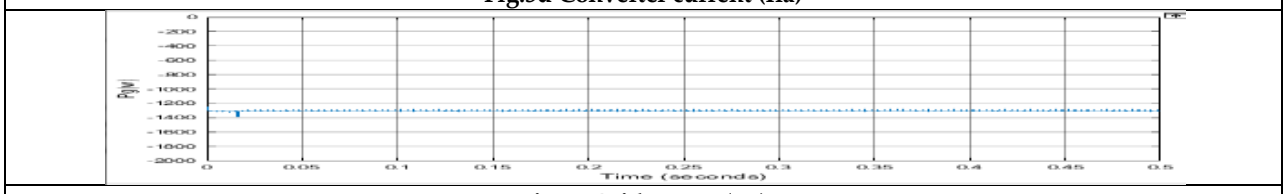


**Fig.3e Grid power (Pg)**

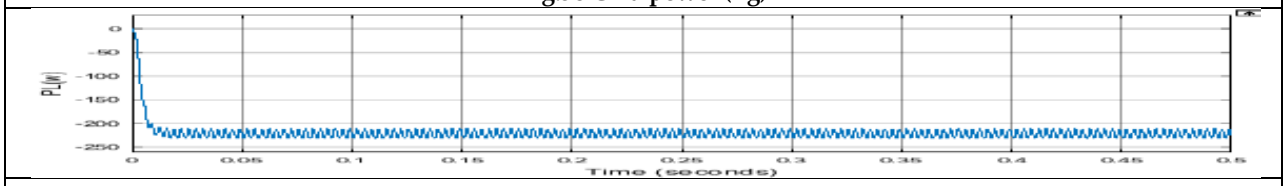


**Fig.3f load power (PL)**

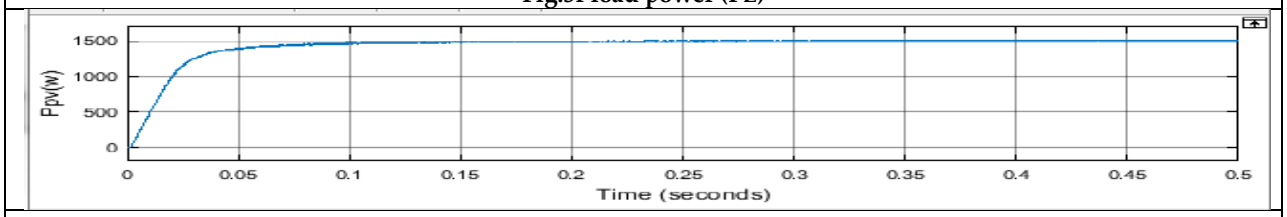


**Fig.3g. Solar PV power (Ppv)**

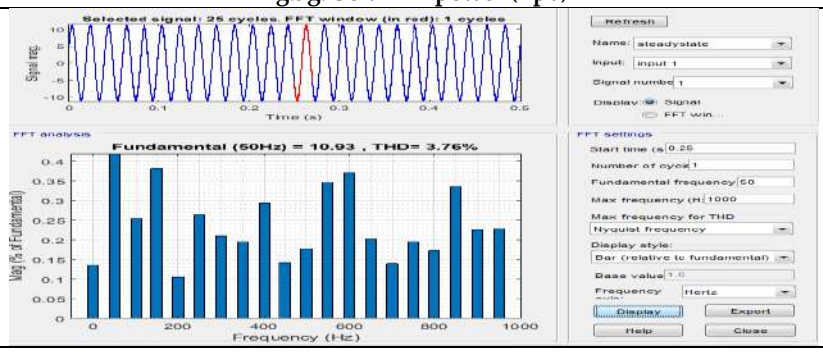


**Fig.3h. THD plot of the grid current**

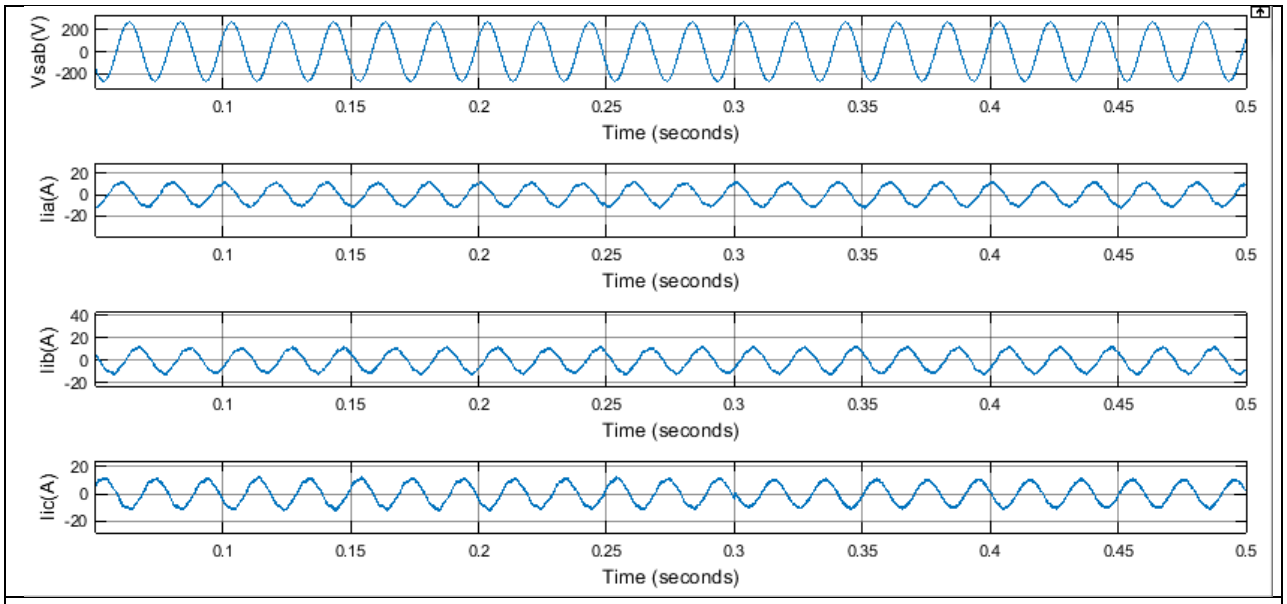


**Fig. 4 (a) line voltage (Vsab) and Converter currents (Iia, Iib, and Iic)**

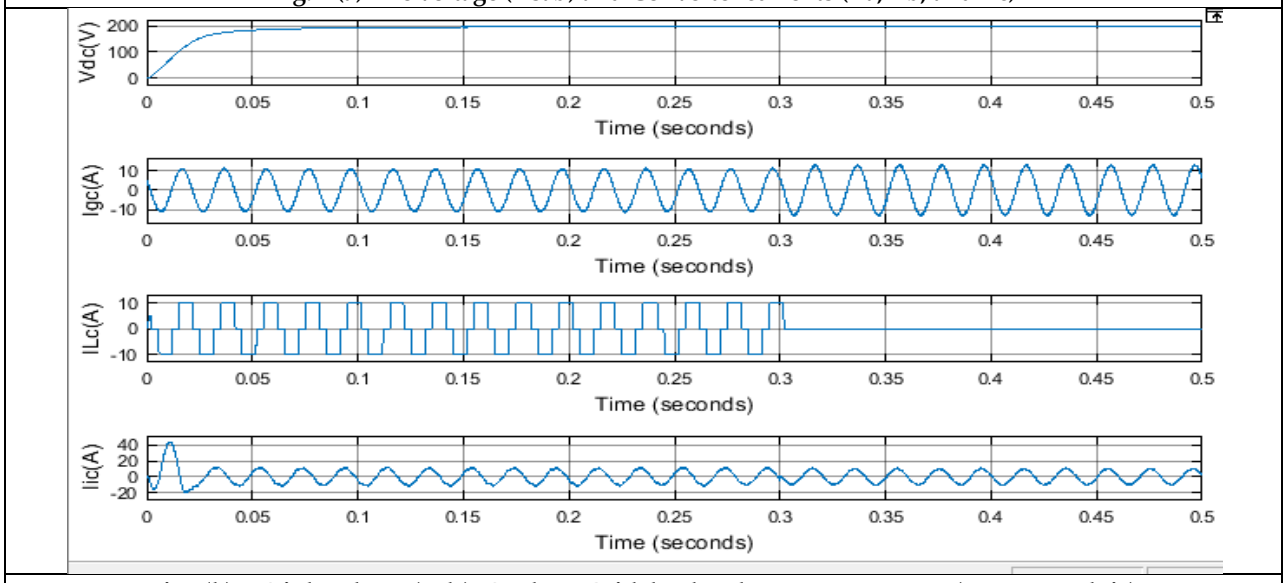


**Fig 4(b) DC ink voltage (Vdc), C- Phase Grid, load and converter currents (Igc, ILc, and Iic)**

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**RESEARCH ARTICLE**

# AI for Waste Management

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**ABSTRACT**

Modern waste management systems are confronted with a myriad of challenges ranging from the increasing volume of waste, diverse waste types, to the need for efficient sorting and recycling mechanisms. The integration of Machine Learning (ML) can significantly augment the capabilities of these systems, ensuring better categorization, optimization, and predictive analysis. This study involve into the design and implementation of an advanced waste management system underpinned by ML techniques. Our proposed system capitalizes on image recognition and classification models to automatically differentiate waste types (e.g., plastics, organics, metals). This aids in the efficient sorting at waste collection and processing centers. Additionally, by analyzing waste generation patterns, predictive models forecast future waste volumes, enabling municipalities and organizations to optimize waste collection schedules and routes.

**Keywords:** Smart Bins, Predictive Analytics, Data collection, Image Recognition, IoT (Internet of Things), Machine Learning, Remote Monitoring, Customer Engagement, Natural Language Processing (NLP)

## INTRODUCTION

Artificial intelligence-based technologies like intelligent garbage bins, classification robots, models, and wireless detection enable the monitoring of waste bins, predict waste collection, and optimize the performance of waste processing facilities. Conventional garbage bins solely collect waste, and sanitation workers must carry out manual inspections to assess the trash level in the bins. This approach is not efficient for routine waste disposal inspections.

Moreover, due to the frequent filling of the containers, disease-causing organisms and insects tend to breed on them (Noiki et al. 2021). Therefore, designing intelligent garbage bin monitoring systems to manage garbage is essential in constructing smart cities. Garbage classification is strongly recommended for municipal solid waste managing and using robots can substantially enhance the efficiency of garbage classification. However, robots require advanced visual and operational skills to function in highly heterogeneous, complex, and unpredictable industrial environments for garbage. Sensor-based waste monitoring is a technology that utilizes sensors to track the amount of waste generated, identify the sources of waste, and measure the effectiveness of waste management strategies in a specific area. Wireless sensor network is a network composed of many self-organized wireless sensors installed in the network to monitor the physical or environmental parameters of the system (Gurram et al. 2022). As illustrated in Fig. 2, a typical wireless sensor network architecture for solid waste treatment systems includes various sensors, such as temperature, humidity, odor, infrared, gas, and sound sensors. Increase waste management efficacy.

## LITERATURE SURVEY

Title: "Optimizing Waste Collection Routes Using Artificial Intelligence: A Comprehensive Review"
Authors: Smith, J., et al.
Summary: Explore studies on AI algorithms applied to optimize waste collection routes, minimizing costs and environmental impact.
Title: "Internet of Things (IoT) Applications in Waste Management: A Literature Review"
Authors: Johnson, M., et al.
Summary: Examine the integration of IoT and sensor networks in waste management, focusing on real-time monitoring and data collection.

**Data Collection and Preprocessing**

Data collection and preprocessing are fundamental stages in implementing AI for waste management systems. To begin, it is essential to clearly define the data requirements, encompassing various aspects such as waste composition, collection schedules, and operational details. The data can be sourced from an array of outlets, including IoT sensors on smart bins, historical records, surveys, and geospatial data from satellites. Quality assurance measures, such as sensor calibration and data validation, ensure the accuracy and reliability of the collected information. In the subsequent data preprocessing phase, cleaning procedures address outliers and missing values, while normalization and standardization techniques bring uniformity to numerical features. Feature engineering may involve creating new variables to enhance the model's predictive capabilities, and data encoding is employed to convert categorical variables into a format compatible with AI models. Consideration of temporal aspects, such as time stamps and seasonal trends, alongside spatial considerations like geocoding and clustering, further enrich the dataset. Ethical considerations, including privacy safeguards and community awareness, play a crucial role in the responsible use of data. Robust documentation, including metadata and a data dictionary, ensures clarity and transparency throughout the process. Continuous monitoring, evaluation, and version control contribute to an adaptive and efficient AI system for waste management.

## METHODOLOGY

The methodology for incorporating AI into waste management encompasses a systematic approach designed to improve the efficiency and sustainability of waste-related processes. Beginning with a clear definition of the problem at hand, the process involves an extensive literature review to glean insights from existing AI applications in waste management. Subsequently, data collection strategies are employed, utilizing sensors, surveys, and other methods to gather both real-time and historical data. The collected data undergoes thorough preprocessing, involving cleaning, normalization, and feature engineering, preparing it for the training of selected AI algorithms. These algorithms, ranging from traditional machine learning models to deep learning architectures, are chosen based on the specific objectives of waste management enhancement. Integration with Internet of Things (IoT) devices, such as smart bins,

enables real-time data input and feedback. Rigorous testing, validation, and subsequent deployment in real-world waste management environments follow, with continuous optimization addressing any identified challenges or limitations. Scaling up the solution and ongoing monitoring and maintenance are integral to ensuring sustained effectiveness. Ethical considerations, community engagement, and transparent documentation further contribute to the successful implementation of AI in waste management, fostering both technical efficiency and responsible, community-oriented practices.

**Model Evaluation**

In the realm of waste management enhanced by AI, model evaluation is a pivotal phase ensuring the effectiveness and reliability of the implemented systems. After the AI model is trained using the preprocessed data, it undergoes a rigorous evaluation process to assess its performance against predefined objectives. Various metrics are employed, depending on the specific goals of the waste management initiative, such as accuracy, precision, recall, and F1 score. These metrics enable quantifiable measurement of the model's ability to correctly predict and classify waste-related patterns. Additionally, validation against separate datasets aids in gauging the generalizability of the model beyond the training set. Continuous monitoring and feedback mechanisms are integral, allowing for adjustments and improvements to be made over time.

**Deployment on a Website**

The deployment of an AI model for waste management on a website is a strategic process aimed at providing users with an accessible and user-friendly tool to optimize waste-related decision-making. This begins with the development of a web application using frameworks like Flask or Node.js, serving as the platform for hosting the AI model. Integration of the model into the backend involves creating API endpoints to handle user requests and deliver predictions. The website's user interface is designed to facilitate easy interaction, allowing users to input relevant waste data. Backend logic processes this input before sending it to the AI model, which then produces predictions or recommendations. Thorough testing ensures the website functions seamlessly, accommodating various waste-related scenarios. The deployment to a server, often utilizing cloud services for scalability, is accompanied by robust security measures to protect user data. Ongoing monitoring and maintenance are crucial, ensuring the system's reliability and efficiency. User feedback becomes an integral part of the iterative process, guiding refinements and enhancements to both the website and the underlying AI model. This deployment strategy aims not only to streamline waste management processes but also to empower users with valuable insights and tools for sustainable waste practices.

**Website Features**

Designing a website for AI waste management involves incorporating features that facilitate efficient waste-related decision-making and enhance user engagement. The website should offer an intuitive user interface, allowing users to input data seamlessly. Feature-rich forms or interactive elements can capture information about the type, quantity, and other relevant details of waste. Integration with the AI model enables users to receive personalized insights, such as optimized waste collection schedules, recycling recommendations, or real-time monitoring of waste levels

Visualization tools, such as charts or graphs, can be implemented to provide a clear overview of waste trends and patterns. Additionally, the website could include educational content on sustainable waste practices, fostering user awareness. Social engagement features, such as forums or community boards, encourage users to share experiences and insights. Mobile responsiveness is vital for accessibility, allowing users to engage with the platform on various devices. Integration with mapping tools can enhance the user experience by visualizing waste collection routes or pinpointing recycling centers. Lastly, incorporating a feedback mechanism empowers users to contribute to the website's improvement and ensures ongoing relevance and effectiveness in addressing waste management challenges. In summary, a well-designed website for AI waste management should seamlessly blend user-friendly interfaces, personalized insights, educational resources, and social engagement features to create a comprehensive platform for sustainable waste practices.

## RESULTS AND DISCUSSION

The results and discussion phase in the application of AI for waste management is pivotal for evaluating the performance and impact of the implemented systems. Upon the deployment and utilization of the AI model, quantitative and qualitative data are collected to assess its effectiveness. Quantifiable metrics such as accuracy, precision, and recall are utilized to measure the model's predictive capabilities, providing insights into its overall performance. These metrics are critical in determining how well the model aligns with the predefined objectives of waste management optimization, whether in route planning, waste categorization, or other targeted areas. The discussion phase delves deeper into the contextual understanding of the results, addressing the practical implications and limitations of the AI system. It involves an exploration of how well the model aligns with the intricacies of real-world waste management scenarios, considering factors such as the dynamic nature of waste composition and collection patterns. Stakeholder feedback and user experiences are also incorporated, providing valuable qualitative insights that contribute to a holistic evaluation. Moreover, the discussion phase offers a platform to consider potential improvements and adaptations to the AI model. It explores avenues for refining algorithms, incorporating additional data sources, or enhancing the interpretability of the model's outputs. Ethical considerations, such as the fairness and transparency of decision-making processes, are examined to ensure the responsible deployment of AI in waste management. In essence, the results and discussion phase is a critical juncture that goes beyond numerical metrics, providing a comprehensive understanding of the AI system's real-world implications, limitations, and opportunities for refinement in the dynamic landscape of waste management

## CONCLUSION

In conclusion, the integration of artificial intelligence (AI) into waste management systems presents a transformative solution with the potential to revolutionize how we handle and optimize waste-related processes. Through the deployment of advanced algorithms and data-driven insights, AI contributes to more efficient waste collection, sorting, and recycling practices. The results obtained from the application of AI models in waste management showcase promising advancements in route optimization, predictive analytics, and real-time monitoring. These outcomes, backed by quantitative metrics and user feedback, affirm the positive impact on operational efficiency and environmental sustainability. Looking ahead, the future of AI in waste management holds exciting possibilities. Further research and development can refine existing models, explore innovative applications, and address emerging challenges. Collaborative efforts between the technology sector, waste management professionals, and local communities are essential to ensure the widespread adoption of AI solutions that align with sustainable practices.

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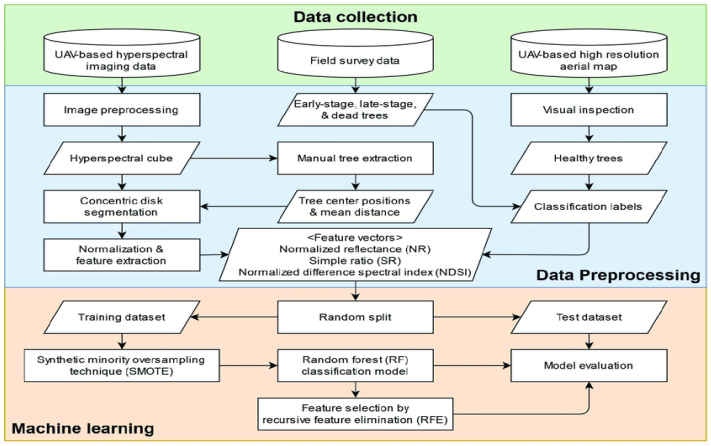


**Fig.1. Data Collection**

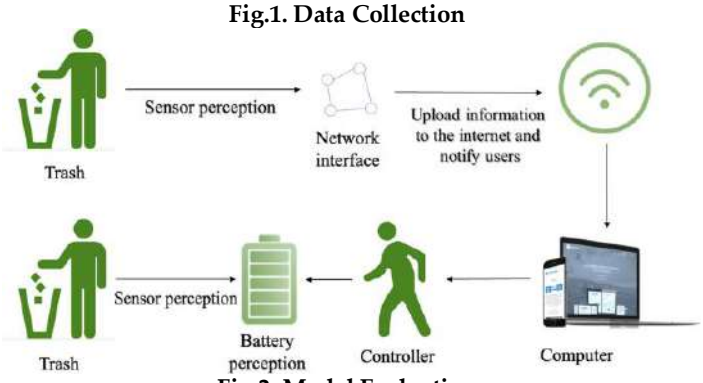


**Fig.2. Model Evaluation**

**Figure 3. Implementation Image**

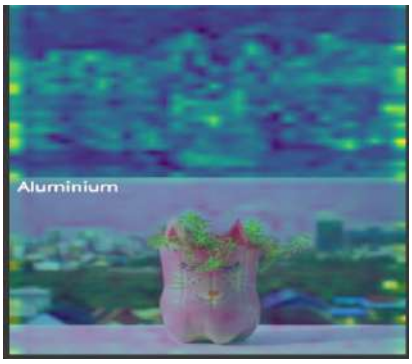


**Figure 4. Aluminum image**

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**RESEARCH ARTICLE**

# Determinants of Shadow Education: Exploring the Impact of Socio-Economic Status, Parental Education Levels, Cultural Expectations, and Policy Environments

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**ABSTRACT**

This paper explores the multifaceted determinants influencing the proliferation and intensity of shadow education. Through an extensive review of existing literature, we identify and analyze key factors including socio-economic status, parental education levels, cultural expectations, and policy environments. Our findings suggest that higher household income and parental educational attainment are strongly correlated with increased participation in shadow education. Additionally, societal and cultural norms, particularly in East Asian countries, exert significant pressure on students to engage in supplementary tutoring. The study also examines the impact of educational policies, such as high-stakes examinations and the perceived inadequacy of formal schooling, on the demand for shadow education. By understanding these determinants, this research aims to provide insights for policymakers and educators to address educational inequalities and improve the overall efficacy of both formal and informal educational systems. Future research directions and policy implications are discussed to foster a more equitable and inclusive educational landscape.

**Keywords:** Shadow Education, Socio-Economic Status, Parental Education Levels, Cultural Expectations, Policy Environments

## INTRODUCTION

In recent decades, the global landscape of education has witnessed a significant phenomenon termed "shadow education," referring to supplementary private tutoring outside formal schooling systems. This widespread practice has sparked considerable scholarly interest due to its implications for educational equity, learning outcomes, and

social stratification (Bray, 2013; Zhang & Bray, 2016; Bray, 2023; Bray & Zhang, 2023; Gupta & Zhao, 2023; Nuryana et al., 2023). Shadow education encompasses a range of informal, often commercially driven educational activities aimed at enhancing students' academic performance, particularly in contexts where traditional schooling is perceived as insufficient (Zhang & Bray, 2016; Tan & Liu, 2023; Bray & Hajar, 2024; Yoo, 2024). The determinants influencing the demand for and provision of shadow education are multifaceted, encompassing socio-economic factors, parental aspirations, cultural norms, and institutional factors such as school quality and curriculum pressure (Stevenson & Baker, 1992; Bray & Lykins, 2012). This paper aims to explore and synthesize existing literature on the determinants of shadow education, focusing on both demand-side and supply-side factors that drive its prevalence across different socio-economic contexts. Understanding these determinants is crucial for policymakers and educators seeking to address educational inequalities and optimize the effectiveness of formal schooling systems (Park et al., 2013). By examining empirical studies and theoretical frameworks, this paper seeks to illuminate how factors such as income levels, parental education, perceived educational quality, and cultural values interact to shape the dynamics of shadow education. Furthermore, the impact of globalization and digital technologies on the evolution of shadow education practices will be considered, highlighting the adaptation of traditional tutoring models in an increasingly interconnected world (Zhang & Bray, 2017; Kobakhidze, 2018).

By synthesizing current research findings, this study provides valuable insights into the multifaceted socio-economic and educational dynamics that influence the widespread growth of shadow education across the world. Ultimately, it seeks to provide insights into potential policy interventions and educational reforms that can mitigate the adverse effects of shadow education while harnessing its potential benefits for students and educational systems (Buchmann et al, 2010; Zhang & Bray, 2017; Holloway & Kirby; Gorkturk & Tulubas, 2021; Xiang, 2021). The interaction of these factors indicates that the growth of shadow education is a complex and multifaceted issue, requiring a depth understanding of various socio-economic, cultural, and policy-related influences. Researchers and policymakers must consider these dimensions to address the implications of shadow education on equity and access within the broader educational context

## REVIEW OF LITERATURE

The growth of shadow education, which refers to private supplementary tutoring outside the mainstream education system, is driven by various socio-economic, cultural, and educational factors. At the forefront is socio-economic status; families with higher incomes can more easily fund private tutoring, prioritizing it to boost their children's academic performance, thereby widening the educational gap across different social segments (Burgess, 2016; Zwier et al., 2021; Habyarimana et al., 2023; Du, 2024). The competitive nature of modern education systems also drives the demand for shadow education, as parents and students seek to gain an edge in high-stakes examinations and admissions processes (Dang & Rogers, 2008). Cultural attitudes towards education, particularly in Asian countries, where academic success is highly valued and seen as a gateway to better socio-economic prospects, further bolster the proliferation of private tutoring (Zhan et al, 2013). Additionally, the perceived inadequacies of mainstream education systems, including large class sizes, insufficient resources, and a lack of individualized attention, compel parents to seek supplementary tutoring as a means to bridge gaps and enhance learning outcomes (Baker et al, 2001). Policy environments also shape the landscape of shadow education, with some governments' regulatory measures and public education policies indirectly fostering the growth of private tutoring markets (Kim & Lee, 2010). Moreover, globalization and the resulting emphasis on global competitiveness have intensified the pressure on students to excel academically, thus increasing reliance on supplementary education services (Entrich, 2015; Matsuoka, 2015; Mohmud, 2021; Zhang, 2021; Gupta, 2022). The rise of technology and online learning platforms has also made private tutoring more accessible and widespread, enabling a broader demographic to partake in shadow education (Zhang, 2019). Urbanization and the migration of families to cities for better educational opportunities further contribute to the proliferation of shadow education, as urban areas typically offer more and varied tutoring services compared to rural regions (Byun & Park, 2012). The intersection of these factors illustrates a complex and

multifaceted phenomenon driving the growth of shadow education globally, reflecting broader trends in educational priorities, socio-economic status, Culture expectation etc.

**Significance of the Study**

The study on shadow education, or private supplementary tutoring, holds significant relevance in contemporary educational discourse. As shadow education becomes increasingly pervasive globally, understanding its determinants and implications is crucial for addressing educational inequalities and enhancing the overall efficacy of educational systems. The findings of this study provide critical insights into the socio-economic, cultural, and policy-driven factors that influence the demand and intensity of shadow education. Firstly, this research highlights the role of socio-economic status and parental education levels in shaping students' participation in shadow education. Higher household income and parental educational attainment are identified as strong correlates of increased engagement in supplementary tutoring (Bray & Lykins, 2012). This underscores the disparities in access to educational resources, suggesting that wealthier families are better positioned to afford additional academic support for their children, thereby perpetuating educational inequalities (Enrich, 2015; Bray, 2021; Cone, 2021; Xiang, 2021). In the second place, this research investigates the cultural and societal factors that lead to a high demand for shadow education, particularly in East Asia. The pressure to excel in high-stakes examinations and the competitive educational environment in these regions lead to a heightened dependence on private tutoring (Byun et al, 2018; Saengboon, 2019; Zhang, 2021; Liu, 2022). This cultural phenomenon highlights the intersection of educational practices and societal values, offering a depth understanding of how cultural contexts influence educational behaviors (Tan & Liu, 2023). Moreover, the research examines the impact of educational policies on the proliferation of shadow education. Policies that emphasize high-stakes testing and perceived inadequacies in formal schooling systems contribute to the growing demand for supplementary tutoring (Zhang, 2021; Bray, 2021; Byun et al., 2023). By identifying these policy-driven determinants, the study provides valuable insights for policymakers seeking to create more balanced and effective educational environments. The implications of this research extend beyond academic understanding to practical applications. For policymakers and educators, the findings offer a foundation for developing strategies to mitigate educational inequalities and enhance the inclusivity and effectiveness of both formal and informal educational systems. Future research directions proposed in this study can further inform policy adjustments and educational reforms aimed at fostering a more equitable educational landscape. In summary, this study is significant for its in-depth analysis of the multiple determinants of shadow education. By shedding light on the socio-economic, cultural, and policy factors influencing private supplementary tutoring, this research contributes to the broader effort of addressing educational disparities and improving educational outcomes globally.

**Objectives of the Study**

1. To investigate the impact of household income on the participation rates in shadow education among students
2. To examine the role of parental education levels in determining participation in shadow education.
3. To analyze the cultural expectations and norms that drives the demand for supplementary tutoring.
4. To evaluate the impact of educational policies on the proliferation of shadow education.
5. To explore policy implications and provide recommendations to address educational inequalities related to shadow education.

**Research Questions**

1. How does household income influence the participation in shadow education?
2. What is the relationship between parental education levels and the intensity of engagement in private supplementary tutoring?
3. In what ways do cultural expectations and societal norms affect the demand for shadow education in different regions, particularly in East Asian countries?
4. How do educational policies, such as high-stakes examinations, contribute to the growth of shadow education?
5. What strategies can policymakers and educators implement to mitigate educational inequalities exacerbated by the prevalence of shadow education?

## METHODOLOGY

This conceptual research paper employs an integrative literature review to explore the determinants of shadow education. The methodology involves a systematic and comprehensive examination of existing scholarly works, policy documents, and empirical studies related to shadow education. The primary sources of data include peer-reviewed journal articles, books, conference papers, and reputable online databases such as JSTOR, Scopus, Google Scholar Web of Science, and ERIC. The selection criteria for the literature were based on relevance, recency, and academic rigor, focusing on studies published in the last two decades to ensure contemporary relevance. The analysis follows a thematic approach, categorizing the determinants of shadow education into socio-economic, cultural, educational, and policy-related factors. Each category is critically analyzed to identify patterns, trends, and gaps in the prior literature. By synthesizing findings from diverse sources, this paper aims to provide a comprehensive understanding of the multifaceted determinants of shadow education, offering insights for policymakers, educators, and researchers interested in addressing educational inequalities and improving educational systems.

## FINDINGS

**RQ1: How does household income influence the participation in shadow education?**

Household income significantly influences participation in shadow education. Firstly, higher household income provides families with the financial resources to afford private tutoring services. Families with greater financial means can invest in supplementary education to enhance their children's academic performance or gain competitive advantages in exams (Xue, 2019; Buyruk, 2020; Yoo, 2024). This ability to pay for additional educational support becomes crucial in contexts where public education systems are perceived as inadequate or where academic success is highly valued for future opportunities, such as admission to prestigious universities or securing better jobs (Kirigwi & Maithya, 2016; Bray, 2022; Jha, 2023). Secondly, household income influences the perception of education as a form of investment. Families with higher incomes often view education as a key pathway to social mobility and economic success. They may be more willing to allocate a significant portion of their income towards educational expenses, including private tutoring, viewing it as a strategic investment in their children's future prospects (Liu, 2019; Entrich & Lauterbach, 2019; Ozdere, 2021; Gulomovna, 2022). Moreover, income disparities can exacerbate educational inequalities. Lower-income families may struggle to afford private tutoring, placing their children at a disadvantage compared to their wealthier peers who can access additional educational support. This disparity can perpetuate existing inequalities in educational outcomes, contributing to a cycle where socioeconomic status determines access to educational resources and opportunities (Tsiplakides, 2018; Ozdere, 2021; Yung & Zeng, 2022; Tan & Liu, 2024). In conclusion, household income plays a critical role in shaping participation in shadow education. It not only determines the financial ability to access private tutoring but also reflects broader socioeconomic inequalities in educational opportunities. It is essential for policymakers and educators to understand these dynamics to bridge disparities and foster educational equity across diverse socioeconomic groups.

**RQ2: What is the relationship between parental education levels and the intensity of engagement in private supplementary tutoring?**

The relationship between parental education levels and the intensity of engagement in private supplementary tutoring is a well-documented phenomenon in educational research. Parental education levels often serve as a significant determinant influencing the decision to enroll children in private tutoring programs. This relationship can be understood through several mechanisms. Firstly, higher parental education levels are associated with greater awareness of the importance of education and academic success for their children (Bray & Lykins, 2012; Liu, 2019; Kato & Kobakhidze, 2024). Parents with higher educational attainment tend to have better knowledge about the educational system, including its strengths and limitations. This awareness may lead them to seek additional support, such as private tutoring, to supplement their child's learning experiences and ensure academic success. Secondly, parental education levels correlate positively with socioeconomic status (SES) (Liu, 2019). Families with higher SES often have more financial resources to invest in supplementary educational activities like private tutoring.

These resources enable them to access and afford tutoring services that may not be available or affordable to families with lower SES. Thus, parental education indirectly influences tutoring engagement through its association with economic capability. Thirdly, parental educational aspirations for their children play a crucial role (Tan & Liu, 2023). Parents who possess higher educational qualifications generally hold greater expectations and aspirations for their children's academic success. Some parents may regard private tutoring as a means to elevate their children's academic results and future educational prospects, aligning with their aspirations for social mobility. Empirical studies support these theoretical assertions. For instance, research in diverse educational contexts, such as East Asia and Western countries, consistently shows that children from families with higher parental education levels are more likely to participate in private tutoring (Liu, 2019; Kato & Kobakhidze, 2024). These studies underscore the significant influence of parental education on the intensity of engagement in private supplementary tutoring, highlighting the multifaceted roles of parental awareness, socioeconomic status, and educational aspirations in shaping educational decisions and outcomes for children. In summary, parental education levels are closely linked to the decision and intensity of engagement in private supplementary tutoring, reflecting broader social and educational dynamics within families and societies.

**RQ3: In what ways do cultural expectations and societal norms affect the demand for shadow education in different regions, particularly in East Asian countries?**

The role of cultural expectations and societal norms in East Asia is profound, particularly in shaping the demand for shadow education and educational practices. In East Asia, cultural values emphasizing academic achievement, such as Confucian principles of education and the pursuit of excellence, exert substantial pressure on students to excel academically (Bray, 1999; Bhorkar & Bray, 2018). These cultural expectations foster a highly competitive educational environment where success in standardized tests, such as university entrance exams, is often seen as crucial for future opportunities and social mobility (Stevenson & Baker, 1992). Consequently, parents and students alike perceive shadow education, such as private tutoring or cram schools, as necessary supplements to formal schooling to ensure adequate preparation and competitive advantage (Liu, 2019; Jansen et al., 2021; Yung & Zeng, 2022; McCoy & Byrne, 2024). Moreover, societal norms in East Asia reinforce the belief that educational success not only benefits the individual but also reflects positively on the family and community. This collective orientation underscores the widespread acceptance and utilization of shadow education as a means to fulfill societal expectations of academic achievement (Buyruk, 2020). For instance, in South Korea and Japan, where educational attainment is closely tied to future career prospects and social status, the demand for shadow education is driven by the desire to meet societal norms of success (Christensen & Zhang, 2021). In contrast, in Western societies where individualism and diverse career paths are more culturally endorsed, the demand for shadow education may be less pronounced due to different societal priorities and educational philosophies (Byun et al., 2023). Thus, while cultural expectations and societal norms in East Asia heighten the demand for shadow education by emphasizing academic achievement and social expectations, these factors may vary in influence across different regions depending on cultural values and educational systems. Overall, the interaction between cultural expectations, societal norms, and educational aspirations shapes the demand for shadow education, particularly highlighting its significance in East Asian countries where academic success is deeply intertwined with cultural identity and societal advancement.

**RQ4: How do educational policies, such as high-stakes examinations, contribute to the growth of shadow education?**

The development of shadow education systems worldwide is heavily influenced by educational policies, particularly those associated with high-stakes examinations. High-stakes examinations are pivotal as they often determine students' future academic and career prospects (Bray & Lykins, 2012). These exams are typically highly competitive and can create intense pressure on students to excel, leading to a heightened demand for supplementary education outside the formal schooling system (Bray, 1999). The impact of high-stakes examinations on educational results and opportunities is a key driver behind the growth of shadow education. In many countries, performance in these exams dictates access to prestigious universities or specific academic tracks, which are perceived as pathways to success and social mobility (Dang & Rogers, 2008; Byun et al, 2018; Saengboon, 2019; Zhang, 2021; Liu, 2022). Consequently, students and their families seek additional tutoring and coaching to enhance exam performance and competitiveness

(Bray, 2022; Jansen et al., 2024). Moreover, the structure and content of high-stakes exams often prioritize rote memorization and exam-specific skills rather than deep understanding and critical thinking (Jung-Hoon, 2018; Liao & Huang, 2018). This focus can lead to dissatisfaction with the formal education system among students and parents alike, who perceive it as insufficient for achieving desired exam results (Bray & Lykins, 2012). Furthermore, the proliferation of shadow education is fueled by perceptions of inequity within the formal education system. Wealthier families can afford private tutoring or enrollment in prestigious exam preparation centers, thereby gaining an advantage over economically disadvantaged students who may not have access to similar resources (Stevenson & Baker, 1992). In conclusion, high-stakes examinations embedded within educational policies significantly contribute to the growth of shadow education by shaping educational priorities, intensifying competition among students, fostering dissatisfaction with formal schooling, and perpetuating socio-economic inequalities. These factors underscore the complex interaction between educational policies and the phenomenon of shadow education, highlighting the need for deatailed policy interventions that address both the drivers and consequences of its proliferation.

**RQ5: What strategies can policymakers and educators implement to mitigate educational inequalities exacerbated by the prevalence of shadow education?**

To effectively address the question of mitigating educational inequalities exacerbated by shadow education, policymakers and educators can implement several strategies. Shadow education refers to private supplementary tutoring and coaching that students receive outside formal schooling, often driven by concerns about academic competition and the desire for enhanced educational outcomes. This phenomenon has been identified as contributing to educational inequalities by favoring students from wealthier backgrounds who can afford such services, thereby widening the gap between privileged and disadvantaged students. One crucial strategy involves regulating and monitoring the shadow education sector. By imposing standards and guidelines on tutoring services, policymakers can ensure that these services supplement rather than substitute formal education. Regulation can include mandating qualifications for tutors, ensuring transparent pricing, and requiring tutoring institutions to align their curriculum with national educational goals (Bray, 2009). Integration of shadow education into formal schooling is another effective strategy. This approach involves acknowledging the prevalence of shadow education and incorporating elements of its success strategies into mainstream classrooms. For instance, identifying effective tutoring methods used in shadow education and training teachers to implement these strategies can help level the playing field for all students (Bai et al., 2019; Punjabi, 2019; Kim & Jung, 2019; Gan & Shahrill, 2019). Financial support and subsidies for disadvantaged students can reduce the financial barriers that prevent them from accessing shadow education. Scholarships or vouchers targeted at low-income families can empower students to seek supplementary tutoring, thereby narrowing the gap between socioeconomic groups (Carr & Wang, 2018; Lancker, 2021; Wainwright et al., 2023). Improving the quality and equity of formal education is fundamental to reducing the need for shadow education. By enhancing the quality of teaching, providing additional academic support within schools, and offering personalized learning opportunities, educators can address the root causes that drive students to seek private tutoring (Byun et al., 2018; Guill et al., 2019; Ozdere, 2021; Bar & Guha, 2023). Community and parental involvement are also crucial. By educating parents on the consequences of shadow education and including those in talks on alternative approaches to enhance their children's learning, reliance on private tutoring can be diminished. Community-based initiatives that offer academic support outside school hours can provide viable alternatives to shadow education (Bray & Zhang, 2018; Mahmud et al., 2018; Yu & Zhang, 2022). Research and data collection are essential for understanding the dynamics of shadow education and its impact on educational inequalities. Continuous monitoring and evaluation can inform evidence-based policymaking and ensure that interventions are effective and sustainable (Bray, 2009). In conclusion, addressing educational inequalities exacerbated by shadow education requires a multifaceted approach that combines regulation, integration, and financial support, improvements in formal education, community involvement, and rigorous research. Through the adoption of these strategies, policymakers and educators aim to foster an educational system that promotes fairness, ensuring every student, irrespective of socioeconomic status, can thrive.

**Figure Explanation (Strategies to reduce the expansion of Shadow Education)**

**Improve Quality of Public School**

- **Enhance Teacher Training & Support:** Providing ongoing professional development and support for teachers to improve their teaching skills and effectiveness.
- **Update Curriculum to be More Relevant & Engaging:** Revising the curriculum to make it more interesting and applicable to students' lives, encouraging better learning outcomes.
- **Foster Collaborative Learning Environment:** Creating an environment where students can learn together, share ideas, and support each other's learning.
- **Involve Community & Parents in Education Decision Making:** Engaging parents and the community in the decision-making process related to education to ensure that their needs and perspectives are considered.

**Strengthen Public School System**

- **Increase Funding & Resources for Schools:** Allocating more financial resources to schools to improve infrastructure, resources, and overall quality of education.
- **Reduce Class Size:** Decreasing the number of students per classroom to allow for more personalized attention and effective teaching.
- **Providing Additional Support Services:** Offering extra academic support, counseling, and other services to help students succeed.
- **Engage Parents & Community in School Improvement Efforts:** Involving parents and community members in initiatives aimed at enhancing school performance and student outcomes.

**Regulate Private Tutoring**

- **Set Clear Standards for Tutoring Services:** Establishing guidelines and standards to ensure the quality and effectiveness of private tutoring services.
- **Monitor & Evaluate Tutoring Practices:** Regularly reviewing and assessing private tutoring practices to maintain high standards.
- **Offer Incentives for Transparency & Accountability:** Encouraging private tutoring services to be transparent and accountable in their operations.
- **Support Alternative to Private Tutoring:** Promoting other forms of support and enrichment that can serve as alternatives to private tutoring.

**Educational Implications**

The results of this research offer crucial insights that can shape policy decisions and educational strategies. Firstly, the strong correlation between higher household income and increased participation in shadow education highlights the need for equitable access to quality education. Policymakers should consider strategies to mitigate the disparities in educational opportunities that arise from socio-economic inequalities. To illustrate, providing low-income families with subsidized or free supplementary tutoring could help bridge the gap, ensuring that every student has an equal chance to boost their academic success. (Bray, 2021). Moreover, the study's revelation that parental educational attainment significantly influences the propensity to engage in shadow education suggests the importance of parental involvement in children's education. Schools and educational institutions should develop programs that encourage and facilitate parental engagement, regardless of their educational background. Providing parents with resources and training on how to support their children's learning at home can be a valuable approach (Kim & Lee, 2010). Cultural expectations and societal pressures, particularly evident in East Asian countries, also play a substantial role in the prevalence of shadow education. Educators and policymakers must work towards creating a balanced educational environment that emphasizes holistic development over merely academic achievement. Promoting a more comprehensive curriculum that includes arts, sports, and life skills alongside traditional academic subjects can alleviate the undue pressure on students to excel solely in academics (Ozdere, 2021; Stastny, 2023; Tan & Liu, 2023). The impact of educational policies, such as high-stakes examinations and the perceived inadequacy of formal schooling, on the demand for shadow education underscore the need for systemic reforms. Education systems should aim to reduce the overemphasis on examinations and instead focus on continuous and formative assessments

that provide a more accurate measure of student learning and development. Additionally, efforts to improve the quality of formal education through better teacher training, updated curricula, and adequate resources are essential to reduce the reliance on supplementary tutoring (Bray & Kobakhidze, 2015; Bray et al., 2018; Soeung, 2021; Budyuk, 2022). In conclusion, addressing the determinants of shadow education identified in this study can lead to more equitable and effective educational systems. By implementing targeted policies and practices that address socio-economic disparities, enhance parental involvement, balance educational pressures, and reform assessment systems, policymakers and educators can work towards a more inclusive and supportive learning environment for all students. Future research should continue to explore these areas to provide further insights and recommendations for fostering educational equity and improving overall student outcomes.

## CONCLUSION

The expansion of shadow education, which includes private supplementary tutoring beyond the mainstream school system, is shaped by a blend of socio-economic, cultural, educational, and policy factors, highlighting its intricate and multi-dimensional character. At the socio-economic level, increasing income disparities and the competitive nature of globalized economies have heightened parental anxiety regarding children's future success, driving demand for additional academic support to secure competitive advantages. This phenomenon is particularly pronounced in countries with high-stakes examination systems, where performance on standardized tests significantly impacts educational and career opportunities, thereby fostering a lucrative market for private tutors. Cultural factors also play a pivotal role, with societal values emphasizing academic excellence and educational attainment, particularly in East Asian countries where Confucian traditions valorize education. These cultural expectations compel parents to commit substantial resources to their children's education, often resulting in significant household expenditures on tutoring services. In regions where public schools are underfunded or face systemic issues such as overcrowded classrooms, inadequate resources, and poorly trained teachers, parents turn to private tutoring as a necessary supplement to fill educational gaps and enhance their children's learning outcomes. Educational policies and government regulations, or the lack thereof, also significantly impact the proliferation of shadow education. In some countries, lax regulatory frameworks allow for the unregulated expansion of private tutoring businesses, while in others, stringent policies aimed at curbing the shadow education industry may inadvertently drive it underground, making it less visible but not necessarily less prevalent. Additionally, technological advancements have revolutionized the shadow education landscape, making online tutoring and educational resources more accessible, thus broadening the reach and appeal of private tutoring services. The COVID-19 pandemic further accelerated this trend, as the shift to remote learning highlighted the deficiencies in public education systems and increased reliance on online supplementary education. Peer influence and social networks play a significant role in the proliferation of shadow education, as community members share insights and advice about effective tutoring services, establishing a normative expectation for participation. The interaction between these factors underscores the intricate dynamics driving the expansion of shadow education globally. Confronting the challenges of the increasing shadow education sector necessitates a diverse strategy that encompasses enhancing public education quality and equity, instituting effective regulatory measures, and fostering cultural changes towards a more holistic and balanced educational development. Policymakers must acknowledge the deep-rooted socio-cultural and economic drivers of shadow education and work towards creating educational environments that reduce the necessity for supplementary tutoring, ensuring that all students have equitable access to quality education without the need for additional, often costly, external support.

**Further Research**

The findings from this study highlight critical areas for further investigation to enhance our understanding of the factors and implications of shadow education. Future research should aim to delve deeper into the specific mechanisms through which socio-economic status and parental education levels influence students' engagement in supplementary tutoring. Additionally, there is a need to explore the psychological and social impacts of shadow education on students, particularly concerning stress, mental health, and overall well-being. Another promising area

for future research is the examination of shadow education in diverse cultural contexts beyond East Asia. Comparative studies across different regions and countries can uncover unique cultural and societal impacts on the expansion of shadow education. This contributes to a broader international perspective on shadow education by identifying shared trends and unique methodologies. With the rise of online tutoring platforms and digital learning resources, it is essential to investigate how technology-mediated supplementary education affects accessibility, quality, and educational outcomes. Research in this area could also assess the effectiveness of online versus traditional face-to-face tutoring methods. Policy-oriented research is crucial to evaluate the efficacy of existing educational policies and interventions aimed at mitigating the need for shadow education. Experimental and quasi-experimental designs could be employed to assess the impact of various policy measures, such as curriculum reforms, teacher training programs, and student support services, on reducing dependence on private tutoring. Finally, an interdisciplinary approach involving economics, sociology, psychology, and education can provide a holistic perspective on shadow education.

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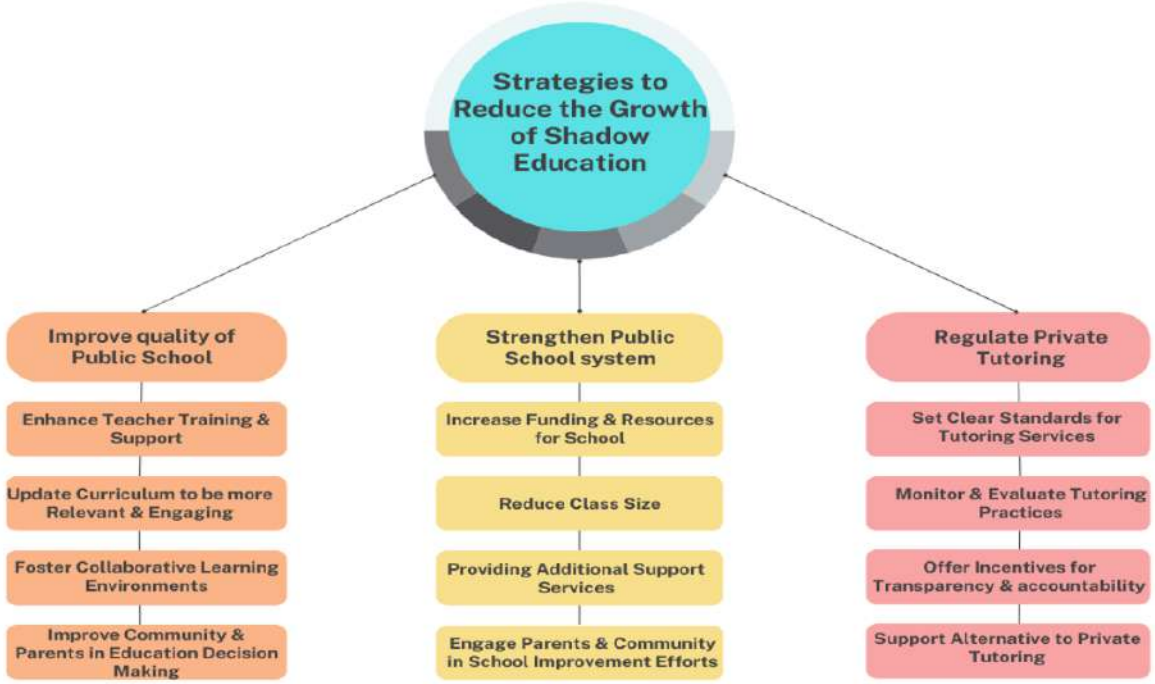


**Figure 1: Strategies to reduce the expansion of Shadow Education**

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**RESEARCH ARTICLE**

# Provision of Data Security using Elliptic Curve Cryptography in Cloud Computing Environment

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## ABSTRACT

Safekeeping of our data being placed in the cloud is always becoming a serious problem and challenging one. The honest reasons for this will be because of the existence of malevolent attacks, data breaches and unsafe contact points. In this juncture, more numbers of investigators offered security mechanisms that includes the components namely access controlling, invasion detection, prevention of occurrences schemes. It is often seen that there are many situations where the attackers have always misused the rights taken by them with the numerous roles in the cloud computing scenario. Hence it would be mandatory to introduce more efficient mechanism for securing the safety of sensitive data which would be placed/recovered in the given cloud. The main philosophies in privacy & security would be none other than the data secrecy and honesty. So, this investigation work heightened a fruitful and better procedure confirming a safer data transmission into the cloud computing atmosphere. The research work provides a additional information security method to cloud that makes use of the Elliptic Curve Integrated Encryption Scheme (ECIES), which confirms that data which could be placed in the Cloud Computing atmosphere is equally trusted and complete. The investigational outcomes prove that the effectiveness of the recommended algorithm results in a strong security level as well as reduces the execution time related to the commonly used existing techniques. Hence in this an Improved Elliptic Curve Cryptography (IECC) algorithm is suggested in this research work which is intended to give better security using modified algorithm. The parameters like encode/decode and store/transfer time are computed and it can be seen that the procedure that is being intended in this research paper take a smaller amount time for all the parameters when compared with prevailing methods. The encoding period of the suggested method is 46 ms with key size=4,096 bits, however the prevailing scheme takes 92 milliseconds for the same. Also,

our proposed methods drastically reduce the deciphering time. All the other parameters presented in the results also certify that our scheme provides better solution when compared with the existing schemes. This reports disclose that the suggested system overtakes the present methods in terms of various factors.

**Keywords :** Cloud computing; Elliptic Curve; Data security; Cryptography.

# INTRODUCTION

Cloud is a technology by which data available in a remote server can be easily retrieved with the internet and it is concealed in nature [1] [2]. It is the On-demand provision of any IT services including servers, storage, databases, any softwares etc. which is normally retrieved over the internet-the so called cloud. In short we can say that any device or gadget which is connected to internet can access the facilities provided. The cloud supports users to right to use the files and applications from any device, since the computing and storage operations happen in the servers in which is placed in a data center, rather than using a local storage. For example, when a user tries to login to his/her Google account form a mobile, tablet, PC, laptop which has internet connection. Hence we say that CC is a State-of-the-art innovation for treating & Relocating data currently in just all possible devices [3]

**SECURITY ISSUES IN CLOUD**

We can also refer to the CIA Triad the information must be set at the focal point of the threesome so as to convey total security of the information. The development of information towards any of the edges of the trio will prompt bargaining the other two properties of information [5].

**Significance of the Study**

Since we are dealing with massive quantity of data, it is extremely compulsory to confirm security. At the same time it is also important to minimize the computational overhead and time complications. Therefore, this research work makes an effort to offer secure and reliable data transmission into the cloud through Modified ECC method. So, the goal of this research was to use the Modified ECC method to enable secure and trustworthy data transfer into the cloud. We will obtain two private keys by splitting the private key (based on size) and encrypting each half individually. That data will be kept online. The key of individual manuscript's encryption is placed into cloud hence only we are able to do the opposite operation for the decryption. The algorithm becomes safer as a result. MECC encryption securely trades data on the unsafe networks by swapping the public keys for encryption. MECC method strongly trades data on the untrusted networks by exchanging public keys for encryption. Clients under no circumstances obliged to disclose or swap their private keys, thereby making it the most reliable encryption strategy is available. If they do, it means that the two secretive Diffie Hellman encoded keys are necessary to decipher single private key obtained in ECC. Lowering the possibility that a cybercriminal would intercept a client's private key at the time of communication. Multi-tenant technique is therefore viewed from the standpoint of the cloud service model in a different way. With the aid of key generation, a Modified Elliptic Curve Cryptography (MECC) is presented to address this issue by safeguarding information which is shared to the multi-tenant environment.

**Problem Statement**

Ongoing studies have diverse difficulties like: computational overheads, time associated with the key creation, clamor/noise responsiveness, difficulties of treating unsized clusters etc. hence disturbing the recovery results from cloud storage. The work intended is endeavored to avoid these impediments through the planned approach.

**Objectives**

The goals associated with the study is referenced beneath:To carry out novel cryptographical procedure for improved ECC that would drastically minimize the key size & extraordinary security thus overcoming existing restrictions like low operational performance, time as well as the computational intricacy.

1. To diminish the scrambling and un scrambling time through firm performance proficiency of suggested method.
2. To rise the accuracy as well as to create alternating keys for security enhancement with the presented scheme.
3. To estimate execution capability of the recommended scheme by means of evaluation criterion namely the encryption as well as the unscrambling time and computation overheads.
4. To relate the success of the proposed model with the other models.

**RELATED WORK**

The research works deliberates the analogous ideas connected to cloud computing, encryption and also the parameters like: decryption, multi-tenant. These works are explored exhaustively alongside their benefits and detriments. One cloud computing structure for multifaceted layers was outlined by [6] security covers three main factors namely the firewall, management of the user's identity and the encryption subject to the growth of business data synchronization& sharing scheme. The benefit is a fundamental technique which gives improved strength for a multifaceted security in the cloud scenario. The difficulty identified was that the assumption structure don't obtain a wrong alarm within the exact period. An infiltration testing was recognized and obstructed a bug is around 99.95 %. Various investigations examined on the combination of cloud and IoT [7] meant to give few characteristics of few characteristics of the cloud. The benefit could be an advanced CloudIoT scheme was recommended to give an improved data between users. The weakness was that the user is necessary to stop and elucidate thorough exploration of cloud computing disputes within the given time-limit. The Mobile cloud scenario was broadly described by [8], and this method carried out to permit the mobile users to get cloud computing advantages by means of a friendly technique for fulfilling the company requests. The gain is simply the vigorous energy-oriented cloudlet-oriented mobile cloud scheme which can be dedicated appropriately to decide over the additional energy usage during the implementation of wireless communication by employing the vigorous cloudlets oriented scheme. Also the weakness is that the wireless connection and the instrument size is restricted. The fluctuating cloud arrangement [9] and the use of this infrastructure through the different service suppliers and the distributing computational profits are placed far away from the DCs. The outcome necessitates a diverse and innovative figuring schemes that could be obtainable by upcoming cloud setup.

Since there are privacy worries, the trustworthy data have to be encrypted prior to the storing in the cloud. Recently we have come across several works in these area have recommended a number of encryption methods. Nonetheless, most of these prevalent works emphasizes only on the secure searching by applying a keyword, and moreover recovers merely Boolean results, which aren't sufficient. For resolve this concern, [10] a solution was presented with a unique and a protected keyword searching mechanism based on Bloom filter that creates the usage through assisting level established on the obtained examination. Another proposed scheme [11] covers three parts: Trusted Authority (TA), Trusted Cloud (TC), and User. This suggestion is meant to provide additional secure scheme to protect the users' data protection, diminish the complication of key creation by means of a modified Identity Based Cryptography (MIBC), and offer data discretion and integrity by means of the Elliptic Curve Integrated Encryption Scheme (ECIES). The advantage is the reduction of time used for key generation and the downside is the key sizes [27].Yet another security structure was suggested [12] containing the access controlling scheme, encipher/decipher procedure and the digital signature method. An innovative Uniqueness centered Elliptic curve Access Control mechanism (Id-EAC). This plus point will be the security structure is delivered with great data safety, manageability and truthfulness for the user data. The downside would be the complexity of the algorithm adapted.

In connection with the data encryption and tenant authentication [13] adopted ECC (Elliptic curve cryptography) due to its least key size. From the investigations it was calculated that the average finishing interval is calculated as 86.076 sec for decryption and 83.153 for encryption. Moreover, the scheme indicated that the scheme deals with a two-layer security and considerably uses smaller storage and key size.There are several works, which discourse about the cloud security [14], data security [15, 16] and secured storage [17, 18]. Among them, [19] concentrated on giving security through reliability relationship rubrics. The confirmation scheme applies the cryptographic calculations for protected storing and recovery.

Another technique namely HSBEE CBC [20] was introduced as an competent data recovery methods. As we can see, the scheme enciphers the data by employing ECC method. Additionally, we observe that the approach used was CBC (Cosine based clustering) technique for grouping of the enciphered information. Also interms of elevating the security, the methodology has appraised the trust for the users retrieving the data., once the trust evaluation process is promising, decryption of the data takes place. Additionally, their methodology has assessed the working of suggested scheme and matched the same with that of the usual schemes.To confirm data security, an innovative practice based on "searchable attribute" based on the encryption was carried out [21]. Another scheme named User Usage Based Encryption (UUBE)[22], built on the exploration encryption technique has been offered as a unique differentiated access control structure.As we can see, a control method for retrieving a multi-faced cloud centered facilities by employing the admission governing model based on various features are presented by [23] and use-case is being presented to examine various cloud services. Subsequent to that, a swapping tokens technique was suggested for extending an exclusive model for the situations of the Inter cloud. Analogous calculations were supported and it can also be confirmed that the suggested one owns improved functionality through the process of taking the total consumers and the associated cloud assets.The reliability and trustworthiness for information existing in the relocation of capacity in multi-facet cloud design was accomplished by [24] through an innovative design in the investigation.

**PROPOSED METHODOLOGY**

The implementation of ECC techniques in scattered computing and dissimilar networking provides substantial usage in inter-reliant networking [25]. ECC is a PKE method built on EC model that produce cryptographic keys faster, lesser as well as highly constructive [26,28]. A basic element is the key strength, that alludes to the intricacy of breaking key and recuperating plain text [26,28]

**Elliptic Curve Cryptography (ECC) Model**

This is an innovative method of cryptographic tool for safeguarding the information in the physical as well as in the cloud storage. It's a superlative practices established in philosophy of elliptical curve. The characteristics of the EC is utilized to produce bases for encryption procedure as a substitute of prevailing techniques which will be using very outsized prime numbers. It operates over the elliptic curve equation for key creation. In the year 1985, N. Kobiltz and V. Miller suggested ECC for changeable data to get safety. The simple clue is to use the elliptic curve to incorporate a distinct logarithm technique [29]. A most important thing of ECC model is that

is always takes tiny key dimensions to give security. When matched with other schemes (key length of 1064) and whereas the ECC use key length of 164 for the identical level of security. It comes under the public-key mechanism and computation shall proceed from a given point on in the elliptic curve to another. EC is treated equal in x-axis and are binary curves. Encryption as well as signature confirmation are carried out with the public key, on the other hand decryption & signature production are completed with private key

**Elliptic Curves**

We assume K remain a field. An EC E over K is specified by Weierstrass equation:

$$y^2 = x^3 + Ax + B \quad (1)$$

For cryptography calculations, we use the equation as

$$y^2 = x^3 + ax + b \quad (2)$$

With the above inputs, we submit the proposed algorithm- Improved Elliptic Curve Cryptography (IECC) algorithm

**Private Key, Public Key and the Generator Point in ECC**

To use ECC, we have to go by the usage of parameters (p,a,b,G,n,h)

p = is a prime number that identifies the magnitude of the given finite field. Here, a & b = are the constants, G is a base point which gives the subgroup of the elliptic curve ,n = number of points , called as the order of the subgroup.
nG = 0
h = cofactor of the subgroup given as a ratio of

$|E| / |Ep| = order of ellipticcurveE|order of EC$ over prime field. $h$(3)

When two individuals connect with each other, individual person should have private and public keys. They would preserve the private key, however public is offered to all. The Private key will be produced through arbitrarily choosing a digit in the range [1 to n-1].
Normally, the Public key is figured out through a calculation, multiplying the private key Ka (ka.G) and a coordinate G (x,y) present in EC. The sender and receiver can thus produce shared secret key (SSK). For an instance, if Ks is taken as the private key of transmitter and Kr indicates private key of the receiver, then we have

Sender's public key (spub)= G * Ks (4)
Recipients public key (rpub) = G* Kr (5)
Both transmitter and receiver will create Mutual Common Key (MCK):
transmitter: MCK = Ks . (Kr.G) (6)
receiver: MCK = Kr . (Ks . G) (7)

***Proposed algorithm***

---

S and R are two persons involved in data transmission
Step 1: Identify an EC
Sender Select an Elliptic Curve:
$E_{Sender}$ **(x coordinate, Y Coordinate)**
Choose a point $G_{Sender}$(Gx,Gy) in the EC
Receiver Select an Elliptic Curve:
$E_{Receiver}$ (x coordinate, Y Coordinate)
Choose a point $G_{Receiver}$ (Gx,Gy) in the EC
Step 2: Take a huge prime number (n)
Step 3: Generate key sets
Sender(S) generating the keys:
Select Private key→$S_{pvt}$
Check → $S_{pvt}$< **n**
Take Point G in EC
Calculate Public Key→$S_{pub}$
Receiver (R) generating the keys:
Select Private key→$R_{pvt}$
Check → $R_{pvt}$< **n**
Take Point G in EC
Calculate Public Key→$R_{pub}$
Step 4: Compute Mutual Common Key MCK→ k
Step 5: Sender and Receiver Exchanges public keys over a channel
Sender share his/her EC and Public key with Receiver
Sender to Receiver→$E_{Sender}$ **(x coordinate, Y Coordinate)**
Sender to Receiver→$S_{pub}$
Receiver share his/her EC and Public key with Sender
Receiver to Sender →$E_{Receiver}$ **(x coordinate, Y Coordinate)**
Receiver to Sender →$R_{pub}$

Step 6: Encrypt → Encode file using key of ECC
Step 7: Output → Decode output from step 6 with generated key and ECC
---

## RESULTS AND DISCUSSIONS

The suggested method's outcome is evaluated based on the metrics presented below. Performance investigation are analysed with various factors namely

1. **Encryption Time** –Time required to encode data
2. **Decryption Time--**Time required to decode data
3. **Interval for upload / download**—Time needed for storing/retrieving

**Encryption/Decryption Times:**
The time taken for decoding /encoding the data is given by these terms. As we can see from Table 1 and Figure 4, we can easily infer that the time taken for encryption and decryption in our model gives minimum time. We also compare the encryption time of our proposed scheme with that of MECC Algorithm suggested by S. Udhaya Chandrika et.al [30] . Tables 2 &3, figures 5&6 clearly shows that the encoding and decoding time in our proposed scheme is far better than that of the MECC method. Hence, we can clearly state that the methodology we have suggested is yielding better results

**Uploading/Downloading times:** This is termed as the amount of time required for storing data into the cloud and retrieving data from the cloud storage. As we can notice from tables 4,5 and figures 7,8, it is clear that our scheme takes a minimum time for both upload and download when compared with that of the MLS architecture [31].

On matching with the MECC [30] and MLS [31] schemes, our proposed scheme using the elliptic curve cryptography has a lesser encoding/decoding times as well as storage/retrieval time. Henceforth, the security-wise and execution speed, our scheme is producing an efficient results.

## CONCLUSION

In this digital era, every individual mainly depends on cloud service providers for keeping their data. In the investigation an innovative security design established on ECC is offered for shielding the end user's data using an elliptical curve procedure. Here, when, a cloud user demand for the access of sensitive data, the suggested technique ensures that the data is always secure. If the invaders are exasperating to crack the data, the same cannot be done since they cannot identify the keys exchanged between the sender and the receiver before the transmission. The performance of the suggested scheme is assessed by means of the parameters namely encoding time, decoding time, and storage effectiveness of data. The algorithm performance is matched with prevailing schemes such as MECC and LSM. From the investigational result study, the proposed technique is realistic and secure. The proposed architecture accomplishes lesser computational overheads and quicker. It also allows ensure the overall security. In upcoming periods, this may be further extended using advance HECC procedures to expand the safety of data stored in cloud.

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**Table 1: Encryption time**

| File Size (MB) | Encryption time (ms) | Decryption time (ms) | Encrypted file size (MB) | Decrypted file Size (MB) |
|---|---|---|---|---|
| 10 | 4 | 3.7 | 0.834 | 10 |
| 20 | 4.6 | 4.2 | 1.34 | 20 |
| 30 | 5.2 | 4.8 | 2.72 | 30 |
| 40 | 5.6 | 5.4 | 3.67 | 40 |
| 50 | 6.3 | 5.9 | 4.29 | 50 |

**Table 2: Encryption time compared with our proposed method and that of MECC Algorithm suggested by S. Udhaya Chandrika et.al[30]**

| Key length (bits) | Encryption-time (ms) of MECC Algorithm suggested by S. Udhaya Chandrika et.al[30] | **Encryption-time (ms)Our Proposed Scheme** |
|---|---|---|
| 100 | 5 | **4.7** |
| 128 | 8 | **7.2** |
| 256 | 10 | **8.8** |
| 512 | 15 | **12.4** |
| 1024 | 24 | **22.9** |
| 2048 | 39 | **35.2** |
| 4096 | 51 | **47.4** |

**Table 3: Decryption time compared with our proposed method and that of of MECC Algorithm suggested by S. Udhaya Chandrika et.al [30]**

| Key length (bits) | Decryption-time (ms) of MECC Algorithm suggested by S. Udhaya Chandrika et.al [30] | **Decryption-time (ms) Our Proposed Scheme** |
|---|---|---|
| 100 | 11 | **9.7** |
| 128 | 25 | **23.2** |
| 256 | 36 | **34.8** |
| 512 | 51 | **48.4** |

| | | |
|---|---|---|
| 1024 | 63 | **60.9** |
| 2048 | 83 | **81.2** |
| 4096 | 159 | **157.4** |

**Table 4: Uploading time (ms) of MLS Algorithm suggested by GhadahAldabbagh et.al [31] and compared with our proposed method.**

| File Size (KB) | Uploading-time (ms) of MLS Algorithm suggested by GhadahAldabbagh et.al [31] | Uploading-time (ms) Our Proposed Scheme |
|---|---|---|
| 10 | 1995 | 1837 |
| 20 | 3156 | 3022 |
| 30 | 4018 | 3874 |
| 40 | 5095 | 4934 |
| 50 | 5894 | 5730 |

**Table 5: Downloading time (ms) of MLS Algorithm suggested by GhadahAldabbagh et.al [31] compared with our proposed method.**

| File Size (KB) | Downloading time (ms) of MLS Algorithm suggested by GhadahAldabbagh et.al [31] | Downloading time (ms) Our Proposed Scheme |
|---|---|---|
| 10 | 1266 | 1158 |
| 20 | 1988 | 1872 |
| 30 | 2146 | 2032 |
| 40 | 3084 | 2911 |
| 50 | 3982 | 3812 |

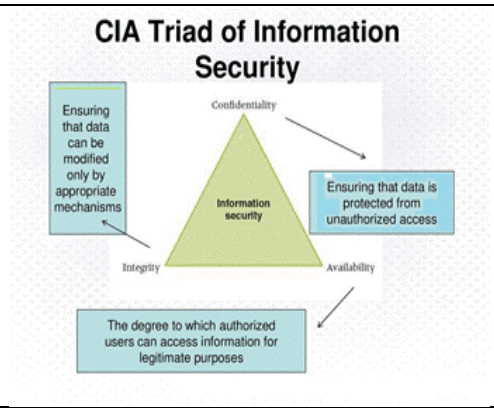


**Fig 1. Security Issues in Cloud**

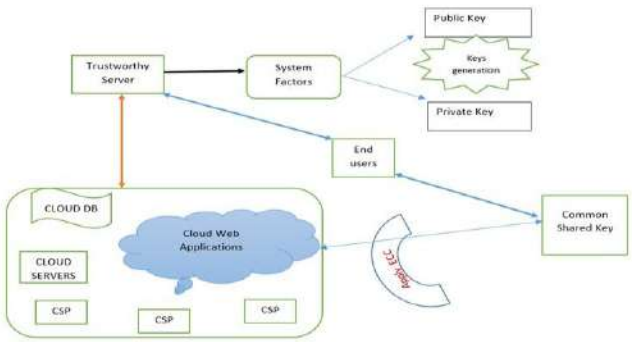


**Fig 2: Outline of validation in web application using ECC**

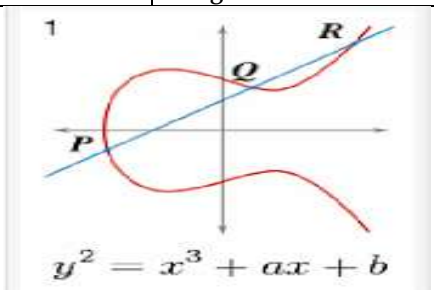


**Fig 3: A typical Elliptic Curve**

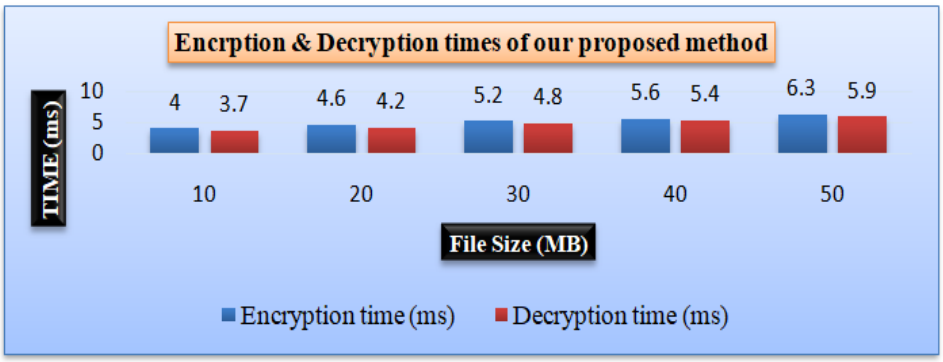


**Figure 4: Comparison of Encryption /Decryption times**

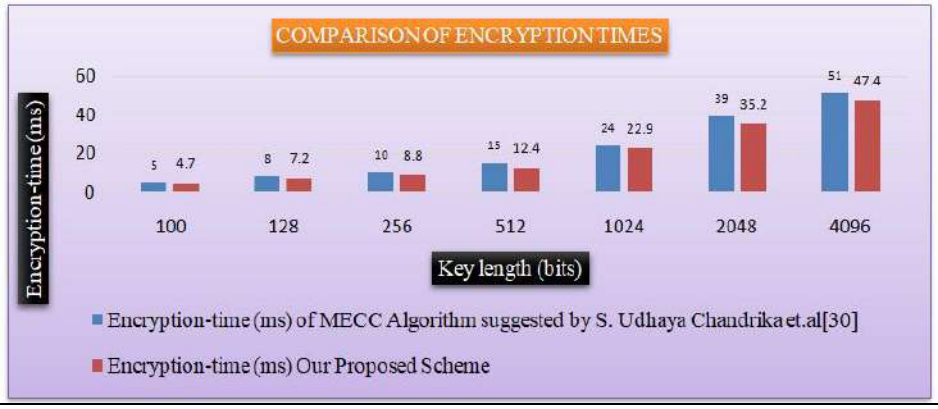


**Figure 5: Comparison of Encryption times**

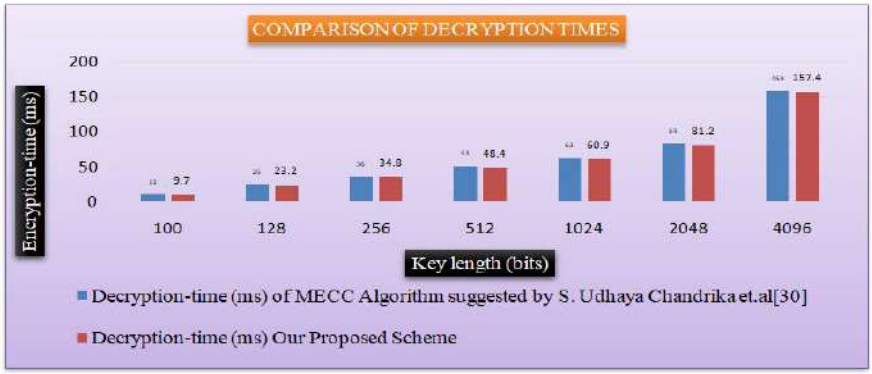


**Figure 6: Comparison of Decryption times**

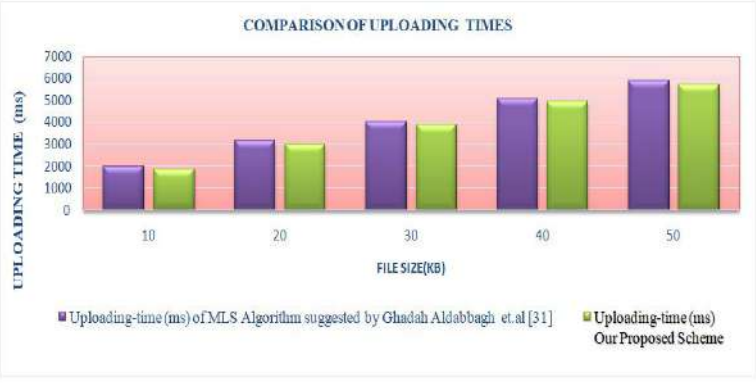


**Figure 7: Comparison of uploading time**

**Figure 8: Comparison of Down loading time**

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**RESEARCH ARTICLE**

# Isolation, Purification, and Characterization of Flavonoid from the Aqueous Extract of the Leaves of the *Phaseolus vulgaris* Linn.

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**ABSTRACT**

*Phaseolus vulgaris* Linn. is native to America but is extensively distributed in warm nations like India. Commonly, it is known as "kidney bean or common bean."The seeds of the plant have undergone extensive study for their medicinal properties, but the leaves of the plant have not been studied much. The aqueous extracts offer significant benefits in terms of health, safety, environmental impact, and practicality. It is crucial to thoroughly study and implement them to create safe, efficient, and sustainable products across diverse disciplines. Therefore, the current aim of the study is to isolate, purify, and characterize flavonoids from aqueous extract of the leaves of the *Phaseolus vulgaris* Linn.Separation of flavonoid achieved by Sephadex LH-20 column chromatography from aqueous extract and enriched by preparative HPLC method. The weight of the isolated compound is 24 mg. The isolated compound was characterized by 1H-NMR spectroscopy, mass spectroscopy,13-carbon NMR, DEPT, and D2O exchange NMR. The spectroscopic analysis gives the structure of a flavonoid as rutin. Alternatively, it is known as 3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside.

**Keywords:** 13-Carbon NMR, DEPT-135,1H-NMR,Isolation, LC-MS,Rutin,

## INTRODUCTION

Flavonoids, predominantly found in a diverse array of plants used for culinary, medicinal, and cultural purposes, have played a vital role in human health and society since ancient times. The current aim of the study is the isolation, purification, and characterization of flavonoids from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn. This is not only a medicinal plant but also one with proven nutritional value.Its widespread cultivation highlights its

importance as a flexible and priceless commodity to the global community.[1]Flavonoids, including myricetin, kaempferol, and quercetin, and their glycosides, have been identified from the bean*Phaseolus vulgaris* Linn.[2],[3].

Aqueous extraction is better for the environment than organic solvents.To enrich the purity of the compound, several important procedures are involved in the isolation and purification of a flavonoid.Sephadex LH-20 is particularly useful for purification since it has the ability to separate compounds based on their size and polarity. Mainly, secondary metabolites from the plant can be separated, as phytoconstituents in plants have a similar polarity range. Upon good maintenance, resin shows good performance, and its capacity for recycling makes it cost an effective option for the isolation of natural products. Due to its regeneration ability, it has wide applications in research and development, industries, and companies. [4]. To validate the structural and functional properties of a flavonoid, characterization is necessary. In-depth details about the molecular makeup, molecular weight, and arrangement of protons of a compound can be obtained by methods including $^{1}$H-NMR spectroscopy,LCMS, and 13Carbon NMR,DEPT,D2O exchange NMR.[5]

## MATERIALS AND METHODS

**Collection, authentication of plant material**

*Phaseolus vulgaris* Linn. was authenticated by St. Xavier's College, Mumbai. The plant specimen collected from Dapoli was matched with the herbarium, and its corresponding voucher number is D.P.2275.

**Chemicals**

Sephadex LH-20 was obtained from Sigma Aldrich. All the solvents used are HPLC analytical grade.

**Extraction**

An important step in isolating a component from a plant is to produce aqueous extracts using successive extraction techniques. By using a series of increasingly polar solvents, sequential extraction separates substances based on their solubility.

**Procedure**

A total of 50 grams of dried, lyophilized plant powder was precisely measured and placed in a beaker, subsequently the inclusion of 1000 milliliters of pet ether. The mixture was subjected to sonication for approximately 10 minutes. After this period, the plant extract was passed through a Whatman filter No. 1, and the filtrate was concentrated. The weight of the empty filtering vessel and the weight of the vessel containing the extract were recorded. The same procedure was repeated with dichloromethane (DCM), ethyl acetate (EAA), methanol, and water.The yield of other extracts had been disregarded. The crude aqueous extract obtained through lypholization has a yield of 30 %, which is employed for the isolation of a flavonoid.[6]

**Size exclusion chromatography**

Sephadex LH-20, a polysaccharide composed of cross-linked dextran with hydroxypropyl groups, is a versatile gel filtration (size exclusion) chromatography medium employed in biochemical and biophysical research for purifying and separating small molecules, particularly natural products, peptides, and low molecular weight compounds. Its exceptional ability to dissolve in organic solvents and effectively mix with both polar and non-polar solvents like acetone, methanol, ethanol, and water gives it the power to enable the separation of a wide variety of chemicals.[7],[8],[9],[10]

**Procedure**

Initially, LH-20 resin was socked with 100% methanol overnight. After that, it is washed three times with 100% methanol. This helps remove previous sample cleaning from resin. The glass column of dimension 1 ft. × 10 cm was taken for size exclusion chromatography. The glass column was washed with methanol, andcotton was plugged into

the end of the column. Wet the cotton plug with the solvent. Pack the glass column with Sephadex LH-20 resin. Allow the methanol to pass through it. Make sure that column does not get dry.[11]

**Sample Loading**

13 grams of crude aqueous extract were precisely measured and dissolved in methanol. Next, the filtrate was carefully poured into the column without disturbing the bed, allowing the methanol extract to flow continuously through it. The 50-mL test tubes were filled with the collected fractions after adjusting the flow rate to 1 mL/min of the column. The chromatographic analysis of fractions from the column was carried out using RP-HPLC. It was found that fractions 53–78 were pure, with a purity level of 74.88%. These fractions were subsequently combined and concentrated through rotary evaporation, ultimately yielding a final product weighing approximately 475 mg.The purity of the isolated compound was enriched by preparative HPLC column chromatography. The RP-HPLC method and preparative HPLC method adopted for the analysis of fractions are mentioned below:

**RP-HPLC method;**

HPLC using a Waters 2695 separation module coupled with a PDA detector (2996) was implemented. The Hemochrom C18, a 150 mm × 4.6 mm, 5-micron particle size column, was utilized as the stationary phase. A solvent A (acetonitrile, ACN) and solvent B (0.05% trifluoroacetic acid, TFA) gradient program was employed as the mobile phase. The gradient program had a run time of 24 minutes, a delay time of 6 minutes, and the following conditions: 0-20 minutes, 0-100% ACN; 20-24 minutes, 100% ACN; 24-25 minutes, 0% ACN; and 25-30 minutes, 0% ACN. The maximum wavelength of absorption ($\lambda$max) was set at 210 nm, and the flow rate was 1 mL/min.[12]

**Preparative liquid chromatography;**

The 475 mg compound isolated from the Sephadex LH-20 column can be enriched by the preparative HPLC method. The compound was dissolved in 1:1 HPLC water with acetonitrile as an eluent. An analytical workstation was connected to the HPLC system, which is made up of an auto- sampler, a pump, and a photodiode array detector. The column setup included a 250 x 20 mm, 5-micron Supelco C18 reverse-phase column. The flow rate was 18.0 mL/min, and the detection wavelength was set at 210 nm. The autosampler tray was maintained at 35°C, and the column was allowed to reach ambient temperature. The mobile phase for the HPLC was a step gradient of ACN and 0.05% TFA. The flavonoid was detected at 210 nm by the photodiode array detector, and individual peaks were recorded. Fractions were collected and isolated using a fraction collector. The solvent was then removed using a rotary evaporator.

**Characterization of isolated compound**

**LCMS**

The LC-MS/MS system used in the study was an Acquity Triple Quadrupole (TQD) system equipped with Mass Lynx V4.2 software (Waters Corp. UK). The chromatographic conditions for LC-MS analysis were carried out using an Inertsil ODS-3 column (150x4.6mm, 3μ). The column eluent was introduced to the MS inlet through an electrospray ionization source operated in positive polarity with a cone voltage of 30V. The capillary voltage was set at 3 kV, while the source and desolvation temperatures were 150° and 500°, respectively. Nitrogen gas was used as the cone and desolvation gas at flow rates of 50 L h-1 and 500 L h-1, respectively. The collision-induced dissociation (CID) experiments were carried out using argon as the collision gas at a flow rate of 0.19 mL/min, with a collision energy applied in the range of 10 to 12 V and a scan time of 0.2s. The mass spectrometer was operated at unit mass resolution. The mobile phases 'A' and 'B' were 0.05% TFA (AR Grade, Merck) and acetonitrile HPLC grade (Finar), respectively. Separation was achieved using a gradient program with the following time (min)/mobile phase 'B' (%) parameters: 0.01/0, 1/0, 20/100, 24/100, 25/0, and 30/0, with a flow rate of 1 mL/min. The column was maintained at 30°, and the eluent was split 1:4 using a Valco valve, with a small portion sent to the MS and the major portion monitored at UV 210 nm. The injection volume was 10 μl.For the preparation of the diluent, acetonitrile was used. The preparation of analytical solutions involved the creation of a test solution at a concentration of 1000 μg/mL.

**$^{1}$H NMR,13C- NMR and DEPT-135;**

All NMR experiments were undertaken using the Avance III HD 400 MHz NMR Spectrometer, equipped with Top Spin software version 3.5pl6 and a Probe PA BBO 500S1BBF-H-D-05 Z from Bruker Biospin Ltd. (Switzerland). Experiments were thoughtfully conducted at room temperatures to ensure accurate results. The solvent chosen to obtain 1H NMR, 13C NMR, and DEPT-135 spectra was DMSO-D6 (99.8% D, Eurisotope). H1 and C13 spectroscopy give a clear picture of the structure of an unknown compound. H1 spectroscopy provides information about the environment of hydrogen atom; therefore, the identification of the structure of a compound becomes easier. The C13 spectra are less complicated as compare to the H1 spectra. C13 spectroscopy gives details of the carbon skeleton. Combination of H1 and C13 spectroscopy identification of unknown compounds is possible.[13]DEPT spectroscopy provides information about the connectivity of hydrogen atoms to carbon atoms. It is widely applicable to the characterization of natural products. This technique gives a brief classification of the types of the carbon atoms based on the connectivity of hydrogen atoms. DEPT provides phase-sensitive data and enhances the sensitivity of carbon-13 NMR signals, making it easier to differentiate between different carbon atoms.[14]

## RESULTS

**Preparative HPLC analysis**

The advanced technique of preparative HPLC is essential for isolating specific chemicals in large quantities from complex mixtures. Its application is widespread across diverse sectors, including pharmaceuticals, biotechnology, and chemical manufacturing, owing to its exceptional efficiency and accuracy. The elution began with acetonitrile at 0%, gradually increasing to 2%, 4%, 6%, and so on up to 16% for 80 minutes. All experiments were conducted at room temperature, which was maintained at 25 ± 2°. A chromatogram of all the fractions was recorded at a wavelength of 210 nm, serving as a monitoring wavelength. The compound was eluted at 16% for 80 minutes. All fractions were collected in 50-ml test tubes. Following analysis using the analytical RP-HPLC method, fractions ranging from 28 to 35 were combined and concentrated using rota vapor. The final product obtained weighed 24 mg and had a percentage purity of more than 98%.

The preparative chromatogram for the isolated compound is clearly illustrated in Figure 1. According to the chromatogram, the retention time of the compound is 8.09 minutes, it has a single sharp peak,and its percentage area is 100%. The UV absorption spectrum of the compound shows peaks at 255nm and 355nm wavelengths, which is consistent with the UV absorption spectrum of rutin as reported in the literature. Consequently, based on the UV data, it is confirmed that the isolated fraction is rutin.[15],[16],[17],[18],[19]

**Characterization of an isolated compound**

Characterization of an isolated compound can be done by a combination of spectroscopic techniques such as LCMS,1H-NMR, C-13 NMR, D2O, and DEPT-135. By analyzing this data and comparing it with literature, the characterization of a compound can be done.

**LCMS/MS;**

Observed full scan +ESI mode gives m/z value 611.1517 [M+H] + with molecular formula C27H30O16. The following figure represents the structure of rutin. The m/z value matches the reference.[20],[21]The structure of rutin contains a quercetin moiety (benzopyranone ring and benzene ring attached to it) and a sugar moiety (glucose and rhamnose ring) connected by glycoside linkage.[22],[23] The numbering of each carbon is given as shown in the structure for ease of characterization. The spectrum of the compound clearly shows a peak at 611.3 m/z as a molecular ion peak. Loss of rhamnose sugar gives fragments at 468.3 m/z, followed by fragmentation by loss of glucose unit at 313.4 m/z. The benzopyranone gives one fragmentation at 155.1 m/z and another fragmentation at 176.1 m/z. The fragmentation pattern is essential to estimatingthe structure of a compound. The fragmentation pattern obtained is closely matched with the literature. Hence, it is confirmed that the isolated fraction from the aqueous extract of the plant is rutin.[24],[25],[26]

**1H-NMRanalysis and 13C analysis(DMSO d6);**

In chemistry, H1 and C13 NMR spectroscopy are crucial analytical methods for identifying functional groups, elucidating structures,and figuring out molecule conformation and dynamics.[27] H1 NMR is useful for identifying hydrogen environments, assessing purity, and providing quantitative analysis, while C13 NMR offers detailed insights into the carbon skeleton of organic compounds, aiding in distinguishing isomers and supporting H1 NMR data for a complete molecular picture.[28]Both methods are non-destructive, preserving samples for further analysis, and they can be used on a variety of substances, including small organic molecules, polymers, natural products, and complex biomolecules.The isolated compound from the aqueous extract of leaves of *Phaseolus vulgaris* Linn. shows the δ values in parts per million (ppm).[29],[30]Fig: 4 and Fig: 5 represent the NMR and C13 spectra of the isolated compound. Table 1 and Table 2 represent the analysis of 1H and C13 NMR data fora separated compound.

There exists a close match between the chemical shift value produced for an isolated compound and the chemical shift values that exist in the literature. Therefore, it is verified that the isolated substance is rutin.

**D2O Exchange NMR;**

This method identifies and separates exchangeable protons in hydroxyl, amine, and amide groups by replacing them with deuterium from D2O. This alters or removes the signals in the proton NMR spectrum, providing clear evidence of the presence and environment of these functional groups.This method considerably improves structural elucidation, which is necessary to precisely ascertain the molecular structure and dynamics of complex natural products, by verifying the positions of exchangeable protons.[31],[32]

In $D_2O$ Exchange NMR, signals at δ 12.60, 10.84–10.86, 9.68–9.74, 9.16–9.19, 5.29–5.35, 5.07–5.16, and 4.35–4.55 disappeared, indicating that these are exchangeable protons of hydroxyl groups. Thus, the identity of the isolated compound as rutin has been verified.

**DEPT-135 NMR**

This method simplifies the interpretation of complex spectra and aids in accurately estimating molecular structures by clearly revealing the carbon-hydrogen framework, which is critical for comprehending the overall architecture and functional group distribution inside a molecule.[32]Fig: 6 represents the DEPT-135 NMR spectra. The DEPT-135 spectra show $CH/CH_3$carbon has a positive peak while $CH_2$carbon has a negative peak.DEPT spectra provide insight into the environments of the various carbon atom types found in the intricate structure. The DEPT-135 NMR data analysis of an isolated compound is shown in Table 3.

## DISCUSSION

Sephadex LH-20 comes in particle sizes ranging from 20 to 80 microns, impacting chromatographic resolution and flow rate. It efficiently separates substances with molecular weights up to several thousand Daltons, making it ideal for purifying peptides and small organic molecules. Sephadex LH-20 enables high-resolution separations that are important for isolating closely related compounds or isomers. Furthermore, it is easy to use because it can be packed into different column sizes depending on the scale of separation required, from analytical to preparative.Sephadex LH-20 is useful for the purification and analysis of natural products and other small chemicals in various scientific fields due to its versatility, solvent compatibility, high resolution, and ease of use.[33],[34]

The substance purified using a Sephadex LH-20 column was further purified to a level of over 95% using preparative HPLC. Preparative HPLC is an indispensable method in chemistry and biochemistry for isolating and purifying specific chemicals from complex mixtures. It is vital for producing the pure molecules required for pharmaceutical development, research, and various industrial applications due to its high resolution, accuracy, and ability to handle large numbers of samples. Preparative HPLC ensures the purity and effectiveness of chemicals used in biochemical research, pharmaceutical formulations, and synthetic chemical processes by effectively separating target molecules from contaminants.

Rutin has unique 1H NMR spectrum features. The 1H NMR spectrum, which is usually recorded in DMSO-d6, has several signals between δ 6.0 and 8.0 ppm, which are characteristic of the aromatic protons on the flavonoid rings. δ 4.5 to 5.35 ppm gives a signal from anomeric protons in rhamnose.The chemical shift values δ 3.00 ppm to 4.5 ppm givea signal from the remaining protons from glucose and rhamnose sugar. According to D2O exchange NMR, the chemical shift from δ 9.00 ppm to 12.00 ppm gives signals from protons of exchangeable hydrogen atoms of the hydroxyl group. The DEPT-135 spectra give information about the carbon-hydrogen relationship in the complex structure. There are tenquaternary, fourteen –CH, one $–CH_2$,and one $–CH_3$carbonatoms present. This information helps to establish the structure of rutin.

## CONCLUSION

To the best of our knowledge, isolation of rutin has not been done yet from an aqueous extract of the leaves of *Phaseolus vulgaris* Linn.Other names for rutin include rutoside, quercetin-3-rutinoside, and sophorin.[22]Rutin is one of the many health advantages of flavonoid glycosides; it is notable for its historical and modern importance. Due to its well-established health benefits, it is used in both conventional and modern medicine.[35]Rutin, a substance with a wide range of pharmacological characteristics, has gained lots of attention because of its anti-inflammatory, anti-carcinogenic, and antioxidant effects on the cardiovascular system.[22],[36] The study provides a reliable method for producing high-purity rutin and outlines a strategy to ensure the consistency and reliability of the substance for future pharmacological and biological research. It emphasizes the importance of accurate characterization. The results of this study will contribute to standardizing methods for characterizing and isolating rutin, making it easier to use in various scientific and medical fields. In the future, advances in green chemistry, biotechnology, nanotechnology, and extraction technologies will greatly benefit the isolation of rutin from aqueous extracts. These developments will significantly expand the use of rutin in functional foods, medications, and cosmetics, while also improving its sustainability and extraction efficiency. To fully realize the potential of rutin as a highly valued natural commodity, it is necessary to prioritize environmentally friendly techniques and cutting-edge technologies.

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**Table 1**: **1H NMR data analysis of a compound isolated from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn. (ppm- Parts Per Million)**

| Atom Number (Position) | Chemical shifts (δ ppm) observed | Chemical shifts (δ ppm) Literature | No. of protons |
|---|---|---|---|
| OH-5 | 12.60 (s) | 12.75 (s) | 1 |
| H (6) | 6.19 – 6.20 (s) | 6.1 – 6.2 (s) | 1 |
| OH-7 | 10.84 – 10.86 (d) | 10.75 | 1 |
| H (8) | 6.38 - 6.40 (d) | 6.3 – 6.4 | 1 |
| H (2′) | 7.52 – 7.55 (s) | 7.5 | 1 |
| OH- 3′ | 9.68 – 9.74 (d) | 9.75 | 1 |
| OH-4′ | 9.16 – 9.19 (d) | 9.25 | 1 |
| H (5′) | 6.81 – 6.85 (d) | 6.75 | 1 |
| H (6′) | 7.64 – 7.67 (d) | 7.5 | 1 |
| H (1′′), OH-2′′ | 5.29 – 5.35 (m) | 5.25 – 5.35 | 2 |
| H (2′′ – 6′′), H(2′′′- 5′′′) | 3.06 – 3.72 (m) | 3.0 – 4.0 | 10 |
| OH-3′′, OH-4′′ | 5.07 – 5.16 (m) | 5.0 – 5.1 | 2 |
| H (1′′′) | 4.40 – 4.41 (d) | 4.9 | 1 |
| OH-2′′′, OH-3′′′,OH-4′′′ | 4.35 – 4.55 (m) | 4.0 – 4.5 | 3 |
| H (6′′′) | 0.98 – 1.07 (d) | 0.8 – 1.0 | 3 |

**Table 2: 13C-NMR data analysis of an isolated compound from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn. (ppm- Parts Per Million)**

| Atom Number (Position) | Chemical shifts (δ ppm) observed | Chemical shifts (δ ppm) Literature | No. of Carbons |
|---|---|---|---|
| C (2) | 156.61 | 157.1 | 1 |
| C (3) | 133.29 – 133.48 | 133.7 | 1 |
| C (4) | 177.41 | 177.8 | 1 |
| C (5) | 161.21 | 161.6 | 1 |
| C (6) | 99.98 | 99.1 | 1 |
| C (7) | 164.07 – 164.12 | 164.5 | 1 |
| C (8) | 93.57 | 94.1 | 1 |
| C (9) | 156.34 – 156.42 | 156.9 | 1 |
| C (10) | 103.90 – 103.96 | 104.4 | 1 |
| C (1′) | 121.05 – 121.16 | 121.6 | 1 |
| C (2′) | 115.97 | 115.7 | 1 |
| C (3′) | 144.75 – 144.82 | 145.2 | 1 |
| C (4′) | 148.41 – 148.50 | 148.8 | 1 |
| C (5′) | 116.25 | 116.7 | 1 |
| C (6′) | 121.94 | 122.1 | 1 |
| C (1′′) | 101.99 | 101.6 | 1 |
| C (2′′) | 74.06 | 74.5 | 1 |
| C (3′′) | 76.43 | 76.9 | 1 |
| C (4′′) | 71.09 | 71.0 | 1 |
| C (5′′) | 75.90 | 76.3 | 1 |
| C (6′′) | 67.01 | 67.4 | 1 |
| C (1′′′) | 101.16 | 101.2 | 1 |
| C (2′′′) | 70.54 – 70.60 | 70.8 | 1 |
| C (3′′′) | 70.36 – 70.43 | 70.4 | 1 |
| C (4′′′) | 71.82 – 71.91 | 72.3 | 1 |
| C (5′′′) | 68.28 | 68.7 | 1 |
| C (6′′′) | 17.74 – 17.93 | 18.7 | 1 |

**Table 3:DEPT NMR data analysis of an isolated compound from the aqueous extract of the leaves of Phaseolus vulgaris Linn. (ppm- Parts Per Million)**

| Atom Number (Position) | Chemical shifts (δ ppm) | Nature of Carbon |
|---|---|---|
| C (2) | 156.61 | Quaternary |
| C (3) | 133.29 – 133.48 | Quaternary |
| C (4) | 177.41 | Quaternary |
| C (5) | 161.21 | Quaternary |
| C (6) | 99.98 | -CH |
| C (7) | 164.07 – 164.12 | Quaternary |
| C (8) | 93.57 | -CH |
| C (9) | 156.34 – 156.42 | Quaternary |
| C (10) | 103.90 – 103.96 | Quaternary |
| C (1′) | 121.05 – 121.16 | Quaternary |
| C (2′) | 115.98 | -CH |
| C (3′) | 144.75 – 144.82 | Quaternary |

| | | |
|---|---|---|
| C (4′) | 148.41 – 148.50 | Quaternary |
| C (5′) | 116.26 | -CH |
| C (6′) | 121.95 | -CH |
| C (1′′) | 102.00 | -CH |
| C (2′′) | 74.07 | -CH |
| C (3′′) | 76.43 | -CH |
| C (4′′) | 71.10 | -CH |
| C (5′′) | 75.91 | -CH |
| C (6′′) | 67.00 | $-CH_2$ |
| C (1′′′) | 101.17 | -CH |
| C (2′′′) | 70.55 – 70.62 | -CH |
| C (3′′′) | 70.38 – 70.44 | -CH |
| C (4′′′) | 71.83 – 71.92 | -CH |
| C (5′′′) | 68.29 | -CH |
| C (6′′′) | 17.76 – 17.95 | $-CH_3$ |

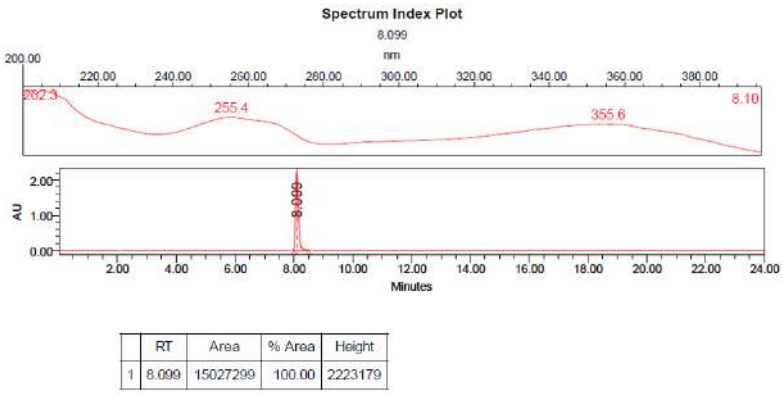


**Fig 1: Preparative HPLC chromatogram of isolated pure compound (24 mg) from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn.**

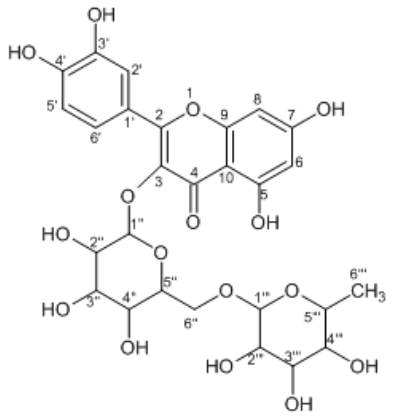

**Fig:2 Structure of rutin (3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside)[37]**

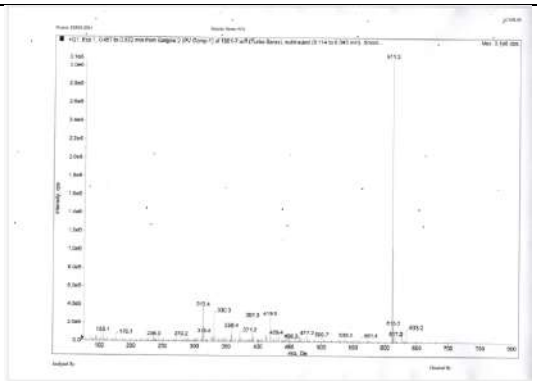

**Figure 3: Mass spectra of a compound isolated from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn.**

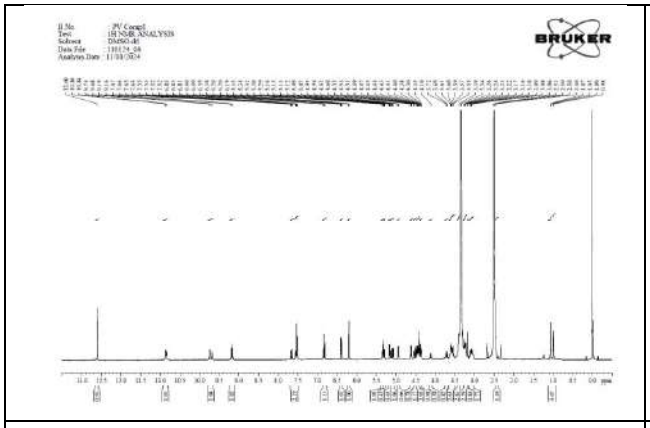

**Fig 4:1H-NMR spectra of a compound isolated from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn.**

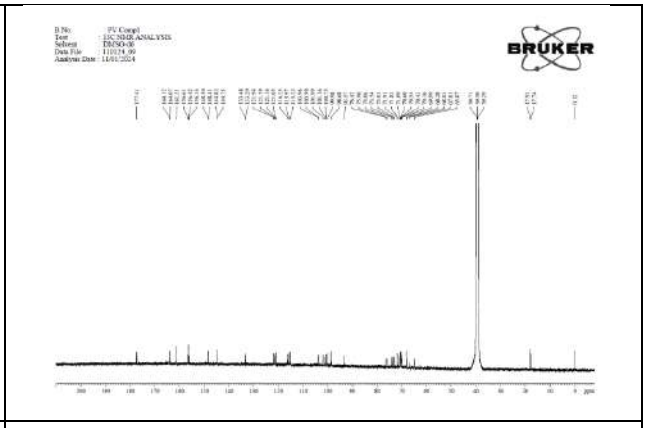

**Fig: 5 13C NMR spectra of the isolated compound from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn.**

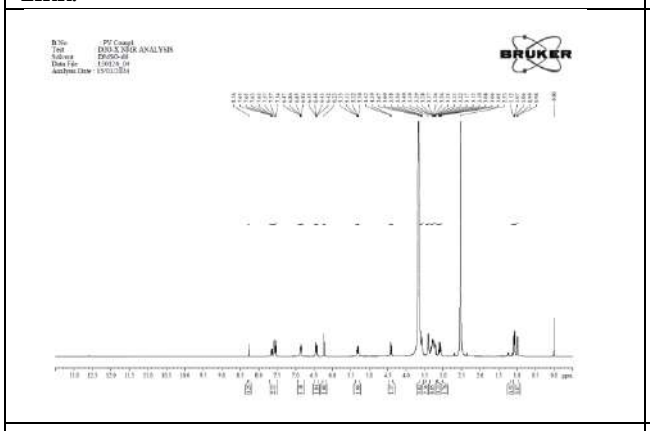

**Fig: 6.D2O NMR spectra of an isolated compound from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn.**

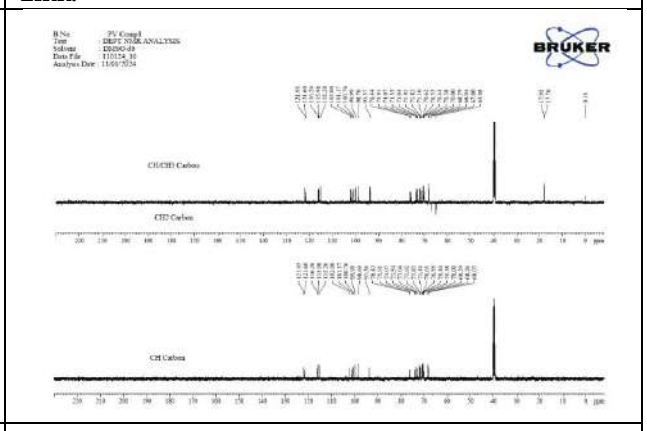

**Fig 7:DEPT-135 NMR spectra of an isolated compound from the aqueous extract of the leaves of *Phaseolus vulgaris* Linn.**

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**RESEARCH ARTICLE**

# A Fractional Order Study of the Type 2 Diabetes Model using the Sumudu Transform Method

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## ABSTRACT

Elevated blood sugar levels serve as prominent indicators of type 2 diabetes, a chronic metabolic condition that poses significant health risks. Genetic predisposition, sedentary lifestyle, poor dietary choices, obesity, and advancing age are among the primary risk factors associated with the development of this disease. Unlike type 1 diabetes, which typically manifests during childhood, type 2 diabetes is characterized by insulin resistance and tends to appear later in life.This paper presents a pioneering mathematical framework for type 2 diabetes (T2D), expanding upon the established SATLP epidemic model. The proposed model categorizes the population into five groups: susceptible individuals (S(t)), affected individuals (A(t)), those under treatment (T(t)), adherents to a healthy lifestyle (L(t)), and those prevented from acquiring the condition (P(t)).In this extended model, we incorporate Caputo's fractional derivative to describe the dynamics of the system, resulting in a set of five fractional differential equations. To obtain approximate analytical solutions for this complex fractional system, we employ the Sumudu transform method (STM), which yields a series solution closely approximating the exact solution.The efficacy of the proposed model is validated through graphical analysis, demonstrating its simplicity and effectiveness in capturing the dynamics of type 2 diabetes. Tables and figures present the results of applying the Sumudu transform method to address the challenges posed by fractional

derivatives in the SATLP model. Through this innovative framework, our objective is to enhance understanding and facilitate the management of type 2 diabetes, thereby contributing to advancements in the field and offering potential solutions for tackling this widespread health issue.

**Keywords:** Type 2 diabetes (T2D), SATLP framework, Caputo's fractional derivative, Sumudutrans form method (STM), Mathematical modelling, Numerical simulation.

## INTRODUCTION

Type 2 diabetes (T2D) is a chronic illness that affects the manner in which the body uses glucose, an essential energy source. In the ones with type 2 diabetes, the body either produces inadequate insulin to control blood sugar levels or responds to insulin less effectively [1].Properlyimplementing lifestyle changes into practice has been shown to dramatically lower the risk of Type 2 Diabetes. These adjustments include eating a balanced diet that is high in fibre and low in fat, getting more exercise, and minimising sitting to maintain a healthy body weight [2-3].Statistical modelling, which makes use of mathematical approaches to analyse and forecast the distribution and transmission of illnesses within an ecosystem, is the core epidemiological framework used for describing the spread of diseases in a population.Several mathematical models, such as those for COVID-19, Ebola, Zika and Lassa, have been developed to mathematically frame the virus models. These models aid in theunderstanding, prediction, and development of control and prevention efforts for the virus [4-7].In the same way, several mathematical models were developed to assess and decrease the effects of diabetes on individuals [8-9].

A novel mathematical SATLP model is being developed to analyze the impact of a healthy lifestyle on the epidemiology of type 2 diabetes (T2D) by creating a comprehensive model and evaluating the effects of treatment rates and the endemic behavior of T2D [10].In epidemiological settings, the severely nonlinear equations have been solved by a number of analytical techniques. In applied fields, a large number of classical differential equations have been solved with the use of analytical methods like theHomotopy perturbation method(HPM), Adomian decomposition method (ADM), and Akbari-Ganji's method (AGM). Additionally, Sumudu transform method (STM), Laplace Adomian decomposition method(LADM), and HomotopySumudu decomposition method (HSDM) have been used to solve fractional differential equations [11-14].Since standard integer-order derivatives are unable to adequately depict a system's long-term behaviour, mathematicians look to the fractional derivative as a useful tool. Fractional derivatives offer a more thorough insight of the behaviour of the system by handling nonlinear and non-proportional influences [15].Sumudu transformation is a mathematical technique that enables the solution of fractionaldifferential equations by breaking them down into simpler, more manageable forms and it does not need any decomposition or perturbation applications. Each ofthese simplified equations can be addressed separately, making the problem-solving processmore efficient and effective [16-19].

Themain objective of this paper is to solve the fractional T2D SATLP model using the powerful analytical technique such as Sumudu transform method (STM). Section 2 provides the preliminary definitions of Caputo fractional derivative and STM. Through the application of the Caputo-derivative, the novel mathematical model for type 2 diabetes is converted from highly classical differential equations to fractional differential equations in Section 3. In section 4, we apply the STM to the strongly SATLP fractional model. We show the model's numerical simulation, tables, and graphs for controlling the disease in sections 4 and 5.

**PRELIMINARIES**

In this section, we define the basic definitions of Caputo-fractional derivative and Sumudu transform method.

**Definition 1: [15]**

For $\alpha > 0$, the Caputo-fractional derivative of order $\alpha$ is defined as follows ($m \geq 1$):

$$c_{D^\alpha} f(x) = \mathcal{J}^{m-\alpha} D^m f(x)$$
$$= \begin{cases} \dfrac{1}{\Gamma(m-\alpha)} \displaystyle\int_0^x (x-\varepsilon)^{m-\alpha-1} f^{(m)}(\varepsilon) d\varepsilon & m-1 < \alpha < m \\ \dfrac{d^m}{dx^m} f(x) & \alpha = m \end{cases}$$

**Definition 2: [19]**

If $F(u)$ is the Sumudu transform of the function $f(t)$, then the Sumudu transform of the Caputo derivative of $f(t)$ of order $\alpha$ is given by

$$S[c_{D^\alpha} f(t)] = u^{-\alpha} \left[ F(u) - \sum_{k=0}^{n-1} u^k f^{(k)}(0) \right], n-1 < \alpha < n$$

Basic mathematical operations of Sumudu transform**[16]** is given below:

1) $S[1] = 1$
2) $S[t] = u$
3) $S[t^{n-1}] = u^{n-1}\Gamma(n), n \in R^+$

**GOVERNING SYSTEM OF EQUATION**

The T2D model **[10]**considers a population of individuals as all adults aged$20-79$, which are then divided into five compartments. All adults are assumed to be potentially susceptible $S(t)$ andthose in this category with healthy lifestyles are designated as $L(t)$. Adults who are affected are classified as $A(t)$, and once they start treatment, they are called $T(t)$. $P(t)$ is a category for those who prevented or recovered health from T2D.

Thus, the classical differential equations of the type 2 diabetes(T2D) model is given as:

$$\begin{aligned} \frac{dS}{dt} &= b - (\mu + \varepsilon + a)S \\ \frac{dA}{dt} &= \varepsilon S - (\tau + \mu + \delta_1)A + \gamma L \\ \frac{dT}{dt} &= \tau A - (\mu + \delta_2)T \\ \frac{dL}{dt} &= aS - (\gamma + \mu + \beta)L \\ \frac{dP}{dt} &= \beta L - \mu P \end{aligned} \tag{1}$$

Initial conditions:

$$S(0) = n_1, A(0) = n_2, T(0) = n_3, L(0) = n_4, P(0) = n_5 \tag{2}$$

where the parameter $\mu$ represents the natural mortality rate, while $b$ represents the birth rate. The parameters $\varepsilon$ and $\gamma$ symbolize the diabetes rates from the susceptible and healthy lifestyle compartments, respectively. The parameter $\tau$ represents the treatment rate, while $a$ denotes the rate of susceptible adults maintaining a healthy lifestyle. Finally, $\beta$ represents the rate at which the healthy lifestyle population transitions to the prevented class. Adults can die from diabetes-related complications in the $A(t)$ and $T(t)$ classes at rates $\delta_1$ and $\delta_2$, respectively.

We reformulate the classical differential equations (1) using the Caputo derivative to obtain a system of fractional ODEs as follows:

$$\begin{aligned} c_{D^{\alpha_1}} S &= b - (\mu + \varepsilon + a)S \\ c_{D^{\alpha_2}} A &= \varepsilon S - (\tau + \mu + \delta_1)A + \gamma L \\ c_{D^{\alpha_3}} T &= \tau A - (\mu + \delta_2)T \\ c_{D^{\alpha_4}} L &= aS - (\gamma + \mu + \beta)L \\ c_{D^{\alpha_5}} P &= \beta L - \mu P \end{aligned} \tag{3}$$

Where $c_{D^\alpha}$ is the Caputo's derivative of fractional order and $\alpha$ is the fractional time derivative

**ANALYTICAL EXPRESSION OF TYPE 2 DIABETES MODEL USING SUMUDU TRANSFORM METHOD (STM)**

In this section, we apply Sumudu transform method for the fractional type 2 diabetes(T2D) model. Taking Sumudu transform for the system (3) with corresponding initial conditions (2), we obtain:

$$\begin{aligned}
S(t) &= n_1 + S^{-1}\left[u^{\alpha_1} S[b - (\mu + \varepsilon + a)S]\right] \\
A(t) &= n_2 + S^{-1}\left[u^{\alpha_2} S[\varepsilon S - (\tau + \mu + \delta_1)A + \gamma L]\right] \\
T(t) &= n_3 + S^{-1}\left[u^{\alpha_3} S[\tau A - (\mu + \delta_2)T]\right] \\
L(t) &= n_4 + S^{-1}\left[u^{\alpha_4} S[aS - (\gamma + \mu + \beta)L]\right] \\
P(t) &= n_5 + S^{-1}\left[u^{\alpha_5} S[\beta L - \mu P]\right]
\end{aligned} \tag{4}$$

The general term of the T2D model is given in equation (5)

$$\begin{aligned}
\textstyle\sum_{n=0}^{\infty} S_{n+1}(t) &= n_1 + S^{-1}\left[u^{\alpha_1} S[b - (\mu + \varepsilon + a)\textstyle\sum_{n=0}^{\infty} S_n]\right] \\
\textstyle\sum_{n=0}^{\infty} A_{n+1}(t) &= n_2 + S^{-1}\left[u^{\alpha_2} S[\varepsilon \textstyle\sum_{n=0}^{\infty} S_n - (\tau + \mu + \delta_1)\textstyle\sum_{n=0}^{\infty} A_n + \gamma \textstyle\sum_{n=0}^{\infty} L_n]\right] \\
\textstyle\sum_{n=0}^{\infty} T_{n+1}(t) &= n_3 + S^{-1}\left[u^{\alpha_3} S[\tau \textstyle\sum_{n=0}^{\infty} A_n - (\mu + \delta_2)\textstyle\sum_{n=0}^{\infty} T_n]\right] \\
\textstyle\sum_{n=0}^{\infty} L_{n+1}(t) &= n_4 + S^{-1}\left[u^{\alpha_4} S[a \textstyle\sum_{n=0}^{\infty} S_n - (\gamma + \mu + \beta)\textstyle\sum_{n=0}^{\infty} L_n]\right] \\
\textstyle\sum_{n=0}^{\infty} P_{n+1}(t) &= n_5 + S^{-1}\left[u^{\alpha_5} S[\beta \textstyle\sum_{n=0}^{\infty} L_n - \mu \textstyle\sum_{n=0}^{\infty} P_n]\right]
\end{aligned} \tag{5}$$

The solutions for $S$, $A$, $T$,$L$,and $P$individuals can be expressed as infinite series, and since the initial conditions are given as: $S_0 = n_1,\ A_0 = n_2,\ T_0 = n_3,\ L_0 = n_4,\ P_0 = n_5$.

When $n = 0$, on comparing both side from the system of equation (5), we obtain:

$$\begin{aligned}
S_1 &= S^{-1}\left[u^{\alpha_1} S[b - (\mu + \varepsilon + a)S_0]\right] = (b - (\mu + \varepsilon + a)n_1)\frac{t^{\alpha_1}}{\Gamma(\alpha_1+1)} \\
A_1 &= S^{-1}\left[u^{\alpha_2} S[\varepsilon S_0 - (\tau + \mu + \delta_1)A_0 + \gamma L_0]\right] = (\varepsilon n_1 - (\tau + \mu + \delta_1)n_2 + \gamma n_4)\frac{t^{\alpha_2}}{\Gamma(\alpha_2 + 1)} \\
T_1 &= S^{-1}\left[u^{\alpha_3} S[\tau A_0 - (\mu + \delta_2)T_0]\right] = (\tau n_2 - (\mu + \delta_2)n_3)\frac{t^{\alpha_3}}{\Gamma(\alpha_3+1)} \\
L_1 &= S^{-1}\left[u^{\alpha_4} S[aS_0 - (\gamma + \mu + \beta)L_0]\right] = (an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{\alpha_4}}{\Gamma(\alpha_4+1)} \\
P_1 &= S^{-1}\left[u^{\alpha_5} S[\beta L_0 - \mu P_0]\right] = (\beta n_4 - \mu n_5)\frac{t^{\alpha_5}}{\Gamma(\alpha_5+1)}
\end{aligned} \tag{6}$$

When $n = 1$, on comparing both side from the system of equation (5), we obtain:

$$\begin{aligned}
S_2 &= S^{-1}\left[u^{\alpha_1} S[b - (\mu + \varepsilon + a)S_1]\right] = b\frac{t^{\alpha_1}}{\Gamma(\alpha_1 + 1)} - (\mu + \varepsilon + a)(b - (\mu + \varepsilon + a)n_1)\frac{t^{2\alpha_1}}{\Gamma(2\alpha_1 + 1)} \\
A_2 &= S^{-1}\left[u^{\alpha_2} S[\varepsilon S_1 - (\tau + \mu + \delta_1)A_1 + \gamma L_1]\right] \\
&= \varepsilon(b - (\mu + \varepsilon + a)n_1)\frac{t^{\alpha_1+\alpha_2}}{\Gamma(\alpha_1 + \alpha_2 + 1)} - (\tau + \mu + \delta_1)(\varepsilon n_1 - (\tau + \mu + \delta_1)n_2 + \gamma n_4)\frac{t^{2\alpha_2}}{\Gamma(2\alpha_2 + 1)} \\
&\quad + \gamma(an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{\alpha_4+\alpha_2}}{\Gamma(\alpha_4 + \alpha_2 + 1)} \\
T_2 &= S^{-1}\left[u^{\alpha_3} S[\tau A_0 - (\mu + \delta_2)T_0]\right] = \tau(\varepsilon n_1 - (\tau + \mu + \delta_1)n_2 + \gamma n_4)\frac{t^{\alpha_3+\alpha_2}}{\Gamma(\alpha_3+\alpha_2+1)} - (\mu + \delta_2)(\tau n_2 - (\mu + \delta_2)n_3)\frac{t^{2\alpha_3}}{\Gamma(2\alpha_3+1)} \\
L_2 &= S^{-1}\left[u^{\alpha_4} S[aS_0 - (\gamma + \mu + \beta)L_0]\right] = a(b - (\mu + \varepsilon + a)n_1)\frac{t^{\alpha_1+\alpha_4}}{\Gamma(\alpha_1+\alpha_4+1)} - (\gamma + \mu + \beta)(an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{2\alpha_4}}{\Gamma(2\alpha_4+1)} \\
P_2 &= S^{-1}\left[u^{\alpha_5} S[\beta L_0 - \mu P_0]\right] = \beta(an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{\alpha_4+\alpha_5}}{\Gamma(\alpha_4+\alpha_5+1)} - \mu(\beta n_4 - \mu n_5)\frac{t^{2\alpha_5}}{\Gamma(2\alpha_5+1)}
\end{aligned} \tag{7}$$

Proceeding like this, one can get

$$S(t) = S_0 + S_1 + S_2 + \cdots$$
$$A(t) = A_0 + A_1 + A_2 + \cdots$$
$$T(t) = T_0 + T_1 + T_2 + \cdots$$
$$L(t) = L_0 + L_1 + L_2 + \cdots$$
$$P(t) = P_0 + P_1 + P_2 + \cdots$$

The approximate analytical expressions of fractional type 2 diabetes (T2D) model is obtained by adding the equations (2), (6) and (7):

$$S(t) = n_1 + (b - (\mu + \varepsilon + a)n_1)\frac{t^{\alpha_1}}{\Gamma(\alpha_1 + 1)} + b\frac{t^{\alpha_1}}{\Gamma(\alpha_1 + 1)} - (\mu + \varepsilon + a)(b - (\mu + \varepsilon + a)n_1)\frac{t^{2\alpha_1}}{\Gamma(2\alpha_1 + 1)}$$

$$A(t) = n_2 + (\varepsilon n_1 - (\tau + \mu + \delta_1)n_2 + \gamma n_4)\frac{t^{\alpha_2}}{\Gamma(\alpha_2 + 1)} + \varepsilon(b - (\mu + \varepsilon + a)n_1)\frac{t^{\alpha_1+\alpha_2}}{\Gamma(\alpha_1 + \alpha_2 + 1)} - (\tau + \mu + \delta_1)(\varepsilon n_1 - (\tau + \mu + \delta_1)n_2 + \gamma n_4)\frac{t^{2\alpha_2}}{\Gamma(2\alpha_2 + 1)} + \gamma(an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{\alpha_4+\alpha_2}}{\Gamma(\alpha_4 + \alpha_2 + 1)}$$

$$T(t) = n_3 + (\tau n_2 - (\mu + \delta_2)n_3)\frac{t^{\alpha_3}}{\Gamma(\alpha_3+1)} + \tau(\varepsilon n_1 - (\tau + \mu + \delta_1)n_2 + \gamma n_4)\frac{t^{\alpha_3+\alpha_2}}{\Gamma(\alpha_3+\alpha_2+1)} - (\mu + \delta_2)(\tau n_2 - (\mu + \delta_2)n_3)\frac{t^{2\alpha_3}}{\Gamma(2\alpha_3+1)} \quad (8)$$

$$L(t) = n_4 + (an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{\alpha_4}}{\Gamma(\alpha_4 + 1)} + a(b - (\mu + \varepsilon + a)n_1)\frac{t^{\alpha_1+\alpha_4}}{\Gamma(\alpha_1 + \alpha_4 + 1)} - (\gamma + \mu + \beta)(an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{2\alpha_4}}{\Gamma(2\alpha_4 + 1)}$$

$$P(t) = n_5 + (\beta n_4 - \mu n_5)\frac{t^{\alpha_5}}{\Gamma(\alpha_5 + 1)} + \beta(an_1 - (\gamma + \mu + \beta)n_4)\frac{t^{\alpha_4+\alpha_5}}{\Gamma(\alpha_4 + \alpha_5 + 1)} - \mu(\beta n_4 - \mu n_5)\frac{t^{2\alpha_5}}{\Gamma(2\alpha_5 + 1)}$$

## NUMERICAL SIMULATION AND DISCUSSION

In this section, the fractional model for type 2 diabetes was numerically solved in order to analyse and control the disease. For thetype 2 diabetes model that is taken into consideration in the Caputo-sense,STM is successfully applied.To facilitate the simulations of$S, A, T, L$ and $P$individuals, the parameter values provided in Table 1 and initial conditions$S(0) = 20, A(0) = 10, T(0) = 5, L(0) = 5, P(0) = 5$ are included in the process. The fractional SATLP type 2 diabetes model, illustrated in Figures 1, 2, 3, 4, and 5, exhibits an enhanced level of flexibilityfor a standard initial timeframe of one year and yields distinct outcomes from the different fractional order$\alpha$responseswithin the model. By altering the values of $\alpha = 0.7, 0.8, 0.9, 1$, it is discovered that a better result is obtained.It provides a more effective way to reach the desired level for illness control. In Figure 1 and 2, susceptible class $S(t)$ andaffected class $A(t)$ increases as the fractional values increase, but in Figure 3, treated class $T(t)$ decrease as the fractional values increase. Likewise, in Figure 4 and 5, healthy lifestyle class $L(t)$ andprevented class $P(t)$ also decreases as the fractional values increase.

As the fractional values decreases from $\alpha = 1$, the solution converges towards a steady state, which suggests that the solution will become more efficient with lower fractional values. Subsequently, to assess the accuracy of the solution, a comparison was conducted between the fourth-order Runge-kutta method (RK4) for classical derivative model (1) and the approximate analytical solutions acquired through the Sumudutransform method. The effectiveness of the Sumudutransform method isdemonstrated through the convergence of the values to the exact solution in integer order for fixing the fractional time derivative $\alpha = 1$in equation (8), along with the given parameters is derived in equations (9-13),

$$S(t) = 20 - 5.8740t + 1.155460500t^2 \quad (9)$$
$$A(t) = 10 - 3.0980t + 0.5519012000t^2 \quad (10)$$
$$T(t) = 5 + 5.5710t - 0.9191959000t^2 \quad (11)$$
$$L(t) = 5 + 2.1810t - 1.046223900t^2 \quad (12)$$
$$P(t) = 5 + 1.4310t + 0.3172761000t^2 \quad (13)$$

and its accuracy with RK4are shown in Tables (2-5) for the different time intervals$t = 0.1, 0.5, 1$ in T2D model. Moreover, as depicted in Figure 6, it's evident that the initial peak of affected individuals A(t) occurred within a two-year period. This surge was attributed to an increase in adults transitioning from the susceptible class ε, coupled with a slower treatment rate τ. However, this number subsequently declined as the treatment rate τ increased and the rate of adults becoming affected from the susceptible class ε decreased, as demonstrated in Figures 7 and 8. The primary strategy to mitigate the progression of T2D is to prioritize extensive treatment or to maintain a healthy lifestyle.

## CONCLUSION

The primary focus of this paper revolves around obtaining the approximate analytical solution for the fractional model of type 2 diabetes (T2D) using the Sumudu transform method (STM). By varying the fractional time derivative $\alpha$, we observe a range of outcomes and enhanced flexibility in this model, offering a greater degree of freedom compared to classical models. A thorough comparison is conducted between the analytical solution obtained and RK4. The findings indicate a satisfactory level of agreement for the specified parameter values, thus underscoring the efficacy of STM in handling fractional nonlinear equations. Visual representations and tables are employed to effectively illustrate the method's efficiency and to analyze the disease. Moreover, a healthy lifestyle and prompt therapy can delay or prevent the development of T2D, slow down its progression, and reduce its symptoms through appropriate control strategies and therapies.

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Table 1: Values of the parameters in type 2 diabetes(T2D) model

| Parameters | Values |
|---|---|
| $b$ | 0.621 |
| $\mu$ | 0.0138 |
| $\varepsilon$ | 0.142 |
| $\tau$ | 0.565 |
| $\beta$ | 0.3 |
| $a$ | 0.2 |
| $\gamma$ | 0.05 |
| $\delta_1$ | 0.04 |
| $\delta_2$ | 0.002 |

Table 2: Comparison of Eq. (9) with the RK4 simulation for Susceptible class$S(t)$.

| $\alpha = 1$ | | |
|---|---|---|
| $t$ | STM | RK4 |
| 0.1 | 19.42415460 | 19.36191562 |
| 0.5 | 17.35186512 | 17.02496375 |
| 1 | 15.28146050 | 15.03482000 |

Table 3: Comparison of Eq. (10) with the RK4 simulation for Affected class$A(t)$.

| $\alpha = 1$ | | |
|---|---|---|
| $t$ | STM | RK4 |
| 0.1 | 9.695719012 | 9.695638062 |
| 0.5 | 8.588975300 | 8.579918812 |
| 1 | 7.453901200 | 7.38576100 |

**Table 4: Comparison of Eq. (11) with the RK4 simulation for Treated class $T(t)$.**

| $\alpha = 1$ | | |
|---|---|---|
| $t$ | STM | RK4 |
| 0.1 | 5.547908041 | 5.548020915 |
| 0.5 | 7.555701025 | 7.568671625 |
| 1 | 9.651804100 | 9.75044600 |

**Table 5: Comparison of Eq. (12) with the RK4 simulation for Healthy lifestyle class $L(t)$.**

| $\alpha = 1$ | | |
|---|---|---|
| $t$ | STM | RK4 |
| 0.1 | 5.207637761 | 5.207847479 |
| 0.5 | 5.828944025 | 5.852924375 |
| 1 | 6.134776100 | 6.31529000 |

**Table 6: Comparison of Eq. (13) with the RK4 simulation for Prevented class $P(t)$.**

| $\alpha = 1$ | | |
|---|---|---|
| $t$ | STM | RK4 |
| 0.1 | 5.146272761 | 5.14616002 |
| 0.5 | 5.794819025 | 5.78241250 |
| 1 | 6.748276100 | 6.65620000 |

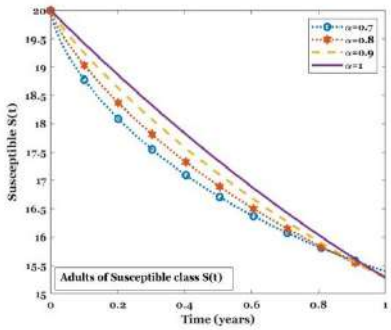


**Figure 1: Plot shows the behaviour of Susceptible class $S(t)$ at different values of fractional order $\alpha$ in T2D model.**

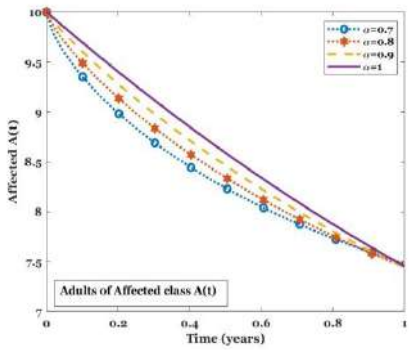


**Figure 2: Plot shows the behaviour of Affected class $A(t)$ at different values of fractional order $\alpha$ in T2D model.**

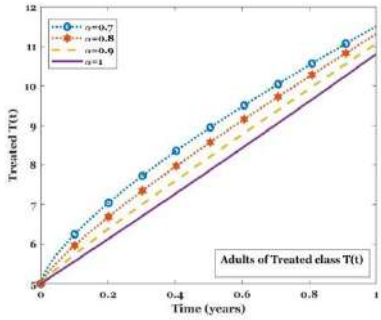


**Figure 3: Plot shows the behaviour of Treated class $T(t)$ at different values of fractional order $\alpha$ in T2D model.**

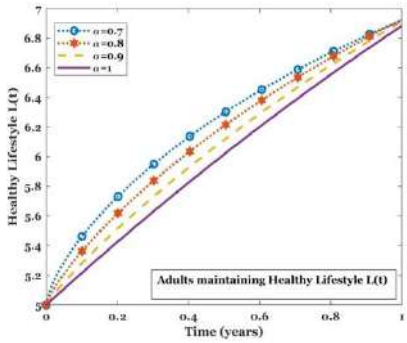


**Figure 4: Plot shows the behaviour of Healthy Lifestyle class $L(t)$ at different values of fractional order $\alpha$ in T2D model.**

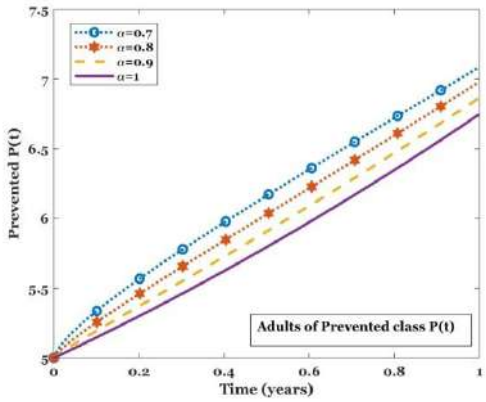


**Figure 5: Plot shows the behaviour of Prevented class $P(t)$ at different values of fractional order $\alpha$ in T2D model.**

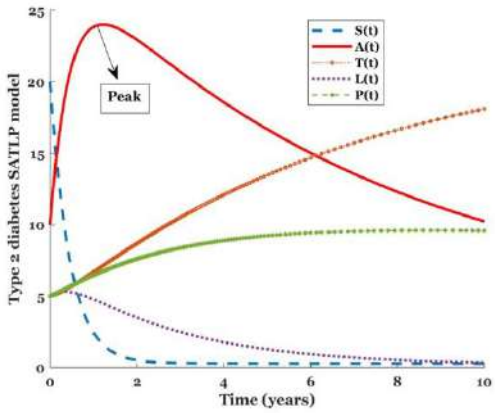


**Figure 6: Affected class $A(t)$ reachespeak within a time frame of 2 years due to the effects of the parameters$\varepsilon = 2, \tau = 0.09$ in the T2D model.**

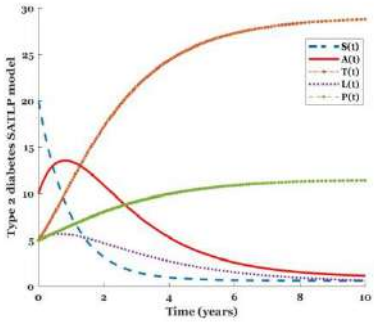


**Figure 7: Treated class $T(t)$ and Prevented class $P(t)$ improves asthe impact of the parameters$\varepsilon = 1, \tau = 0.5$ in the T2D model.**

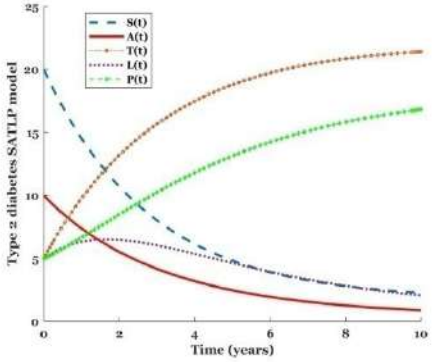


**Figure 8: Visual representation of Treated class $T(t)$ and Prevented class $P(t)$improving over time in the T2D model.**

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**RESEARCH ARTICLE**

# Tannins Analysis from *Rubus fructicosus* Root Extract using High Performance Thin Layer Chromatography for Antidiarrheal Activity

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## ABSTRACT

*Rubus fructicosus* widely used for its pharmacological properties. Considering to known data *R. fructicosus* has the potential to improve health through its antibacterial, antioxidant, neuropharmacological, and DNA damage protective properties. The purpose of the current investigation was to determine anti-diarrheal effect of *R. fructicosus* root extract. Phytoconstitutents present in root analyzed using HPTLC for phytochemical identification and quantification. Tablets were formulated using the direct compression method. Castor oil induced diarrhea model mice were divided into normal control, disease control, marketed ,extract and formulation**.** Each group containing sixswiss albino mice each. Tablets were evaluated for weight variation, friability, hardness, disintegration time , In vitro dissolution found to be in limit. Among formulation F6 batch shown good drug release as compared to other formulations. High Performance Thin Layer Chromatography (HPTLC) shown the presence of gallic acid 0.68 ng in extract and 0.43 ng in formulation. With the help of HPTLC the presence and quantification of tannin was identified and shown the promising antidiarrheal activity when compared with reference.

**Keywords** *R. fructicosus*, HPTLC, Tannins, Castor oil, Antidiarrheal.

## INTRODUCTION

Medicinal plants are vital to human survival and the maintenance of good health because they contain bioactive phytochemicals. *Rubus fructicosus*, more recognisable as the blackberry, is a member of the Rosaceae family. In Hindi, it is also referred to as Kaala jamun. Plant leaves and roots are traditional home treatments for anaemia, irregular menstruation, diarrhoea, and dysentery (1).The roots, which have a long-standing antidiarrheal effect, include tannins and saponin as phytoconstituents (2). High-performance thin-layer chromatography (HPTLC) is a more advanced version of thin-layer chromatography (TLC) since it produces data with a high level of accuracy and precision (3). The current work was carried out to use the HPTLC technique to analyze the phytochemicals present in a *Rubus fructicosus* root extract and to evaluate antidiarrheal activity of extract and formulation.

**Objectives**

Analysis of phytoconstituents present in root extract using HPTLC.
Evaluation of antidiarrheal activity of extract and formulation.

## MATERIALS AND METHODS

**Plant**

*Rubus fructicosus* root extract.

**HPTLC**

Chemicals and solvents :
Methanol , Distilled Water, Toluene , Ethyl acetate , Formic acid.

**Working Standard**

Pure Drug.

**Preparation of Standard Solution**

Weigh an accurately 10mg of Gallic Acid and transfer into 10 ml of volumetric flask. Dissolved in 5 ml methanol and sonicate for 10 min and made up volume up to 10 ml by using methanol to obtain 1000 ug/ml. From this stock solution inject 0.4ul.

**Chromatographic Conditions**

Chromatographic conditions are as follows:
Stationary phase: precoated silica gelHPTLC aluminium plates (10×10 cm, 0.2 mmthick). Mobile phase: Toluene : Ethyl acetate : Formic acid : Methanol ( 4 : 3 : 2 : 1).
Saturation time: 15 minutes.
Wavelength: 271 nm.

The HPTLC analysis was performed in an air conditioned room maintained at 22°C and 55% humidity using precoated silica gel aluminium backed plates (10 × 10 cm, 0.2 mm layer thickness. 0.4 $\mu$L of the standard solutions of Gallic acid were spotted using a Linomat autosampler fitted with a 100 $\mu$m Hamilton syringe and operated with settings of a band length. The plates were developed to a distance using toluene : ethyl acetate : formic acid: methanol (4: 3: 2 : 1 v/v) mobile phase in CAMAG twin trough chamber pre saturated with mobile phase. The developed plates were air dried and scanned with a CAMAG TLC Scanner equipped with winCATS planar chromatography software that was used for spectra recording, and data processing.

**Standard Calibration Curve for gallic acid and linearity**
From 1000ug/ml stock solution 100 to 600 ng/band was applied on HPTLC plate. The plate was developed and scanned as per the chromatographic condition mentioned. The peak areas were recorded. Calibration curve of Gallic acid was prepared by plotting peak area versus concentration.

**Preparation of conventional tablets**
Direct compression procedure: To achieve homogeneous mixing, all the formulation's constituents were sieved through sieve no.25 and mixed in a mortar pestle. The pre-compression properties of the mixed powder were then assessed. Additionally, this blended powder was compacted into tablets using a tablet compression machine and an 8mm flat punch. The parameters for post-compression evaluation for further punched tablets were assessed.

**Antidiarrheal activity castor oil-induced diarrhea method**
**Castor Oil Induced Diarrhea Model**
30 Swiss albino mice of either sex will deprive of food for 18 hours with free access to water and will divide randomly into five groups each containing 3 mice, as mentioned below. Group I as Normal Control Distilled water 10ml/kg (p.o) Group II as Disease Control Castor oil 0.5 ml of (p. o) . Group III as Marketed Loperamide 3mg/kg (p. o) . Group IV as Root Extract 400 mg/kg (p. o). Group V as formulation 200 mg/kg (p. o) .The animals were placed individually into cages in which the floor was lined with transparent paper for the collection of fecal matter. The transparent paper was changed every hour for a total of 4 hours. The mice was then removed from their cages and the weight of feces obtained by subtracting the weight of filter paper from the weight of feces and filter paper. The onset of diarrhea, the number of wet stools, the total number, and the total weight of fecal output noted. Finally, the percentage of diarrheal inhibition, as well as the percentage of the weight of total fecal output, calculated by using the following formula: percentage of diarrheal inhibition = mean number of wet stools (control group − treated group)/mean number of wet stools of the control group x 100 (4,5).
*Grouping of Animals*

**Tablet Evaluation**
Pre-compression study was evaluated for six batches Bulk density , Tapped density , Compressibility index ,

## RESULT AND DISCUSSION

**Characterization by HPTLC** :Linearity of Standard Gallic Acid

**Tablet Evaluation**
Pre-compression study was evaluated for six batches Bulk density , Tapped density , Compressibility index , Hausner's, Ratio and ,Angle of repose (6). Post compression study was evaluated for Appearance , Hardness , Thickness ,Friability , Uniformity of weight, Disintegration , In vitro dissolution (7-11) . Hausner's Ratio and Carr's Index (%) shows excellent property. Angle of repose shows good property. All six formulations were found to have hardness values between 4 and 4.5 kg/cm2. Friability was determined to be between 0.5% and 0.6%, while the thickness was found to be between 3.60 and 3.62 mm. There was a weight variation of 185.1 mg to 217.9 mg. Conventional tablets were found to disintegrate in less than 15 minutes. F6 has shown good drug release as compared to other batches.

**Stool weight**
Stool weight in Water treated group was found to be 86.88 ± 4.21 mg. Treatment with castor oil. significantly ($P<0.001$) increased the stool weight and the stool weight was found to be 189.66 ± 33.49 mg. Treatment with Loperamide, Extract and formulation significantly ($P<0.001$) reduced the stool weight as compared to the standard treatment and the stool weight was found to be 101.95 ± 2.21 mg, 134.73 ± 4.16 and 139.31 ± 3.37 mg respectively.

## CONCLUSION

Characterization and Quantification of Gallic acid was performed using HPTLC. Tablets were prepared using different ratios of excipients. A total of 6 formulations were formulated and evaluated. The bulk density , tap density, angle of repose, carr's index and hausner ratio for the powder was determined and the results were found to be within limit for all formulations. Tablets were prepared by direct compression method. These formulations were evaluated for hardness, weight variation , friability, drug content , disintegration and in-vitro drug release. Formulation F6 found to be the good formulation. The model used to assess the ant-diarrheal activity include castor-oil induced diarrhea, appeared to act inhibition of diarrhea by reducing the absorption of water from the intestines that lead to the increase in defecation. This is due to the presence of tannins present in *R. fructicosus* .The findings in the present study confirm the anti-diarrheal activity of the root extract and formulation of *R. fructicosus* thus provide the scientific basis for the traditional use of this plant in the treatment of diarrhea and its effects.

## ACKNOWLEDGMENT

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**Table 1: Formulation table**

| **Ingredients (mg)** | F1 | F2 | F3 | F4 | F5 | F6 | Category |
|---|---|---|---|---|---|---|---|
| **Extract** | 100 | 100 | 100 | 100 | 100 | 100 | API |
| **Lactose** | 80 | 80 | 80 | 80 | 80 | 80 | Filler and Binder |
| **Magnesium stearate** | 3 | 4 | 7 | 6 | 5 | 2 | Lubricant |
| **Sodium starch glycolate** | 6 | 5 | 2 | 3 | 4 | 7 | Disintegrant |
| **Talc** | 3 | 3 | 3 | 3 | 3 | 3 | Glidant |
| **Potato starch** | 8 | 8 | 8 | 8 | 8 | 8 | Binder |
| **Total weight** | 200 | 200 | 200 | 200 | 200 | 200 | |

**Table 2: Castor oil induced diarrhea grouping**

| Group no. | Groups | Treatment | No.of Animals Swiss albinomice M/FWt. 25-30g |
|---|---|---|---|
| I | Normal Control | Distilledwater10ml/kg(p.o) | 6 |
| II | Disease Control | 0.5mlofcastoroil(p.o) | 6 |
| III | Marketed | Loperamide3mg/kg(p.o) | 6 |
| IV | Root Extract | 400mg/kg(p.o) | 6 |
| V | Formulation | 200mg/kg(p.o) | 6 |
| Total=30 | | | |

**Table 3: Quantification of Galllic acid**

| Phytoconstituent | Extract | Formulation |
|---|---|---|
| Gallic acid | 0.68 ng | 0.43 ng |

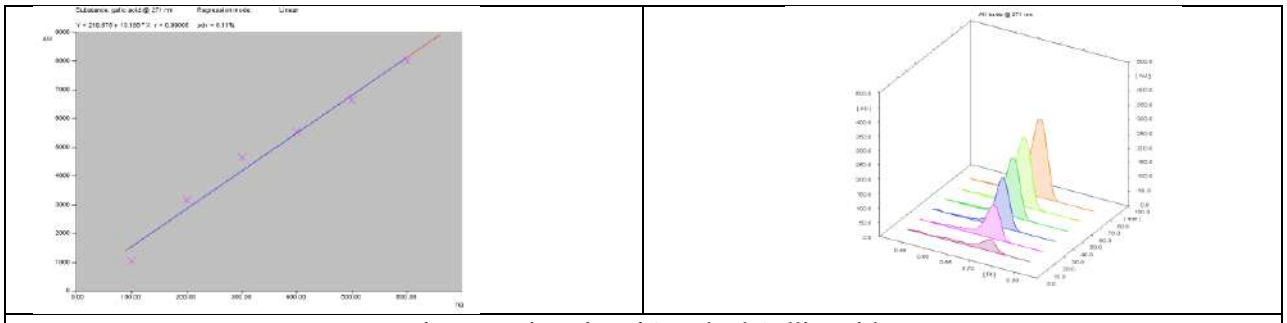
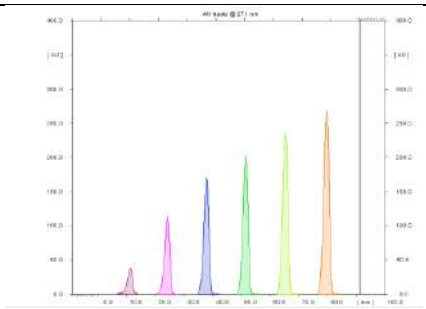


**Fig No. 1 Linearity of Standard Gallic Acid**



**Y=219.675+13.165*X**
**R=0.99005**

**Fig No. 2 3d Standard Gallic Acid**

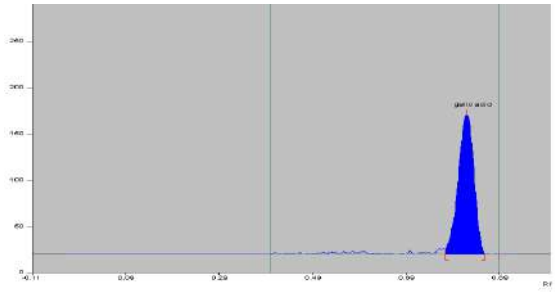


**Fig No. 3 Standard Gallic Acid**

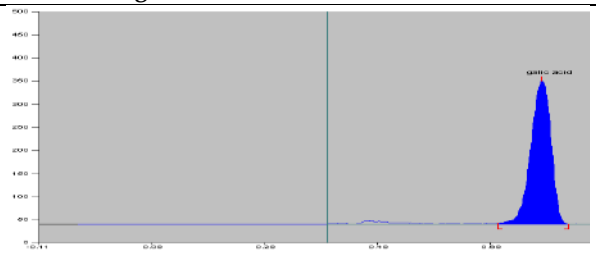


**Fig No. 4 Gallic Acid in extract**

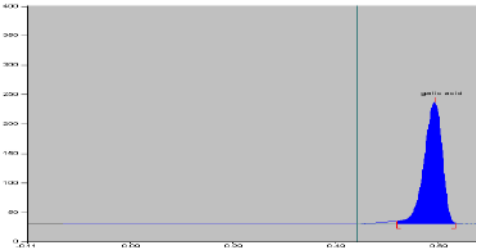


**Fig No. 5 Gallic Acid in formulation**

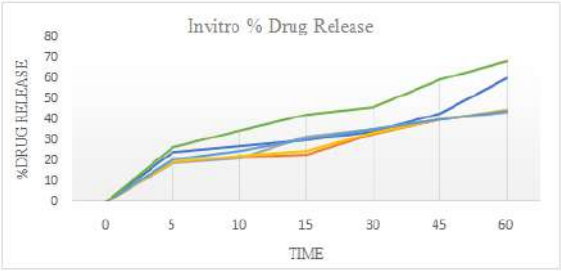



**Fig No. 6 Invitro % drug release**

**Figure 7. Disease stool**

**Figure 8.Extract stool**

**Figure 9. Loperamide**

**Figure 10. Formulation**

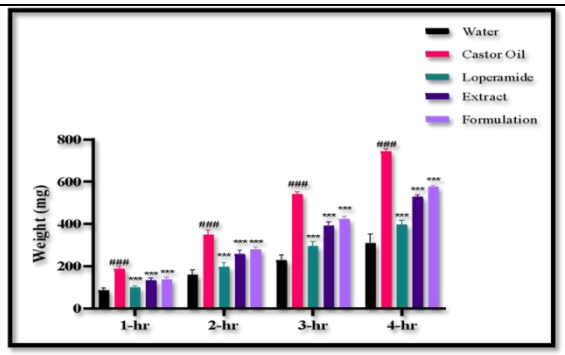



**Figure 11. Effect of treatments on stool weight (mg). Data was analyzed by two way ANOVA followed by Bonferroni's multiple comparison test (n=6). ###p<0.001 as compared to control and *** p<0.001 as compared to standard.**

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**RESEARCH ARTICLE**

# Exploring Living and Working Conditions of Migrant Workers in Construction Industry in Karnataka - an Empirical Analysis

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**ABSTRACT**

The paper analyzes the living and working conditions of migrant workers in Karnataka's construction industry, focusing on their socioeconomic realities and the factors influencing their migration. It aims to investigate the motivations behind these workers decisions to seek employment in this sector, particularly examining the economic conditions in their places of origin, local job opportunities, and the potential for increased wages. The study evaluates the living conditions of these workers, highlighting critical aspects such as health, housing, and economic well-being. Many migrant workers face significant challenges, including inadequate housing, poor sanitation, and limited access to healthcare, which adversely affect their quality of life. The research explores existing policies aimed at improving the living and working conditions of migrant workers, assessing their effectiveness and identifying gaps in implementation. The study identifies challenges faced by migrant workers, including social isolation, exploitation, and lack of access to essential services. By employing a mixed-methods approach, the research gathers comprehensive data through surveys and interviews, providing a nuanced understanding of the migrant experience in Karnataka's construction sector. The analysis seeks to inform policymakers and stakeholders about the pressing needs and rights of migrant workers, advocating for improved conditions and support systems to enhance their livelihoods.

**Keywords:** Migrant Workers, Construction Industry, Challenges, Polici.

## INTRODUCTION

Migrant workers are integral to the construction industry in Karnataka, significantly contributing to its growth and development. In 2021, the construction sector in Karnataka employed approximately 1.5 million migrant workers, highlighting their crucial role in meeting the demand for labor in urban areas. The study examines the factors driving

these workers to seek employment in the construction sector, their living and working conditions, and the policies aimed at improving their circumstances. Economic factors are the primary drivers of migration to Karnataka's construction sector. Many workers are motivated by the prospect of higher wages compared to their home regions, where job opportunities are scarce. For instance, the average daily wage for construction workers in Karnataka ranges from 600 to 800, significantly higher than wages in rural areas of the state. The rapid urbanization and infrastructure development in cities like Bangalore, which has seen a population growth of over 46% from 2001 to 2011, have created a high demand for labor, attracting workers from various parts of Karnataka. For many, the construction sector represents a viable means to support their families and improve their living standards. However, the living and working conditions for migrant workers in Karnataka remain challenging. Many reside in temporary accommodations that lack basic amenities such as clean water, sanitation, and electricity. According to reports, around 70% of migrant workers live in substandard housing, which contributes to health issues, including respiratory problems and other diseases. Migrant workers significantly contribute to India's labor market, with Karnataka being a key destination for interstate migration. According to the Economic Survey 2016-17, Karnataka experienced a net in-migration, hosting approximately 32 lakh migrants from other states, 25 lakh migrants from Karnataka resided in other states. The survey highlights that, on average, around 9 million people migrate between states annually for work or education, with Karnataka's interstate migration rate at 37.82%, notably higher than the national average of 4.48%. Factors driving this migration include the pursuit of better economic opportunities, as many migrants seek to enhance their family income amidst limited job prospects in their native regions. The state's rapid urbanization and demand for labor, particularly in sectors like construction, further amplify this trend, underscoring the complex dynamics of labor migration in Karnataka (Economic Survey 2016-17).

The construction sites often pose safety risks, with workers facing long hours in hazardous environments for low pay. Data from the Karnataka State Building and Other Construction Workers' Welfare Board indicates that accidents on construction sites are a significant concern, with many workers reporting injuries due to unsafe practices. To address these issues, the national and Karnataka government has implemented several policies aimed at improving the living and working conditions of migrant workers. Initiatives include enhancing access to healthcare services and enforcing labor laws to protect workers' rights. The Karnataka Labour Welfare Board has also launched programs to provide social security benefits to construction workers. Despite these efforts, the effectiveness of such policies is often hampered by inadequate enforcement and the informal nature of many construction jobs. Migrant workers in Karnataka's construction sector face numerous challenges, including exploitation by employers, job insecurity, and limited access to social services. Many workers are unaware of their legal rights, which leaves them vulnerable to abuse and discrimination. The ongoing disparities in economic development within Karnataka further complicate their situation, as many continue to migrate in search of better opportunities enduring significant hardships. The construction sector in Karnataka offers employment opportunities for many migrant workers, significant issues regarding their living and working conditions persist. A concerted effort from both government and civil society is essential to ensure that the rights and well-being of these vital workers are prioritized and protected.

**OBJECTIVES**

1. To investigate the factors that prompted the migrant workers to seek employment in the construction sector in Karnataka, with a focus on economic conditions of migrant workers, job opportunities, and potential wage increases.
2. To evaluate the living and working conditions of migrant workers in Karnataka's construction sector, focusing on Health, Housing, and Economic being
3. To explore the policies promotes better living and working conditions of migrant workers in Karnataka.
4. To identify the key challenges and issues faced by migrant workers in Karnataka's construction sector.

**HYPOTHESES**

1. Economic hardship and limited job prospects in their hometowns are primary factors driving migrant workers to seek employment in Karnataka's construction sector.

2. Poor living and working conditions among migrant workers in Karnataka's construction sector had profound impact on their health and economic well-being.
3. Lack of awareness among migrant workers in construction sector on labour rights and inadequate legal protections system paved way for economic exploitation and hardship.

**SAMPLING AND SAMPLE SIZE**

The study employed a combination of purposive sampling and simple random sampling to gather primary data from 66 respondents, for instance migrant workers from Dharmapuri District, chosen to ensure both accessibility and representativeness. The primary focus was on migrant workers currently employed in the construction sector in Karnataka, as this group is often overrepresented in the workforce. Purposive sampling was used to identify the respondents who were already employed in Karnataka's construction sector. A structured Interview Schedule has been utilized to facilitate consistent and comprehensive data collection. The interviews were conducted with respondents to gain insights into the employment practices and working conditions and other issues faced by these workers. The combination of these sampling methods and the structured interview approach aimed to produce a comprehensive and reliable outcome.

## METHODOLOGY

The study adopted both document analysis and survey methods to collect both primary and secondary data, thereby utilizing qualitative and quantitative approaches. The dual approach allowed for quantitative data to facilitate the analysis of primary data through numbers and statistics, qualitative data provided richer context and deeper insights. Document analysis was chosen because it enables the examination of historical records, government documents, and other written sources relevant to the study. Document analysis complements the survey method by offering a broader perspective on the subject, enhancing the overall depth and reliability of the research findings. The survey method was employed to gather opinions and perspectives on the living and working conditions of migrant workers in the construction industry in Karnataka, ensuring a grounded and evidence-based analysis.

**DATA ANALYSIS**

**DEMOGRAPHIC VARIABLES**

The study selected only three demographic variables: marital status (married and unmarried), gender (male and female), and occupation (organized and unorganized). This framework was utilized to explore the living and working conditions of migrant workers in Karnataka's construction sector, aiming to uncover how these factors impact their experiences and challenges.

The table shows the distribution of respondents marital status, gender, and occupation among the 66 participants in the study. Of these, 25 respondents (37.87%) are married, 41 respondents (62.12%) are unmarried, indicating that a majority of the migrant workers in the construction sector are unmarried, which highlights potential implications for their living and working conditions and underscores the need for targeted support services. In terms of gender, 32 respondents (48.48%) identify as male, while 34 respondents (51.50%) identify as female, reflecting a slightly higher representation of female migrant workers in the construction sector and suggesting the importance of addressing gender-specific challenges and needs within this workforce. Regarding occupation, 22 respondents (33.33%) are employed in organized sectors, whereas 44 respondents (66.66%) work in unorganized sectors. The significant majority in unorganized occupations emphasizes the precarious nature of employment for many migrant workers in the construction industry, highlighting the urgent need for improved labor protections and support for this vulnerable group.

**TESTING OF HYPOTHESES AND INTERPRETATION**

The t-test assessing the impact of economic hardship and limited job prospects in respondents' hometowns on migrant workers seeking employment in Karnataka's construction sector, categorized by marital status, reveals

significant differences. Among the 66 participants, married respondents reported a mean score of 10.28 with a standard deviation of 2.29, while unmarried respondents had a mean score of 12.44 (SD=2.89). The t-value of 2.93, with a p-value of 0.05, indicates a statistically significant difference in perceptions of economic hardship and job prospects between married and unmarried workers. This finding leads to the rejection of the null hypothesis and acceptance of the alternate hypothesis, which suggests that marital status influences the perceptions of economic challenges driving migration. The results imply that unmarried workers may be more affected by economic conditions in their hometowns, potentially motivating them to seek employment in Karnataka's construction sector more urgently than their married counterparts. Unmarried migrant workers are more prevalent in Karnataka's construction sector due to socio-economic factors. Many young individuals migrate for better job opportunities amid economic hardship and limited prospects in their hometowns. Unmarried workers, often younger and less burdened by familial responsibilities, are more adaptable and willing to relocate for work. They seek independence and improved socio-economic status, which is often unattainable in their native areas. The construction sector's demand for labor, combined with fewer family commitments, makes it easier for unmarried individuals to accept jobs that involve long hours and challenging conditions, highlighting the need for targeted support policies.

The t-test evaluating the impact of poor living and working conditions on the health and economic well-being of migrant workers in Karnataka's construction sector, categorized by gender, reveals significant differences. Male respondents reported a mean score of 12.41 with a standard deviation of 2.22, while female respondents had a mean score of 18.09 (SD=4.06). The t-value of 3.19 and a p-value of 0.01 indicate a statistically significant difference in perceptions of economic hardship and health impacts between genders, leading to the rejection of the null hypothesis. This shows that female migrant workers may experience greater adverse effects from poor conditions due to factors such as increased vulnerability, higher exposure to health risks, and fewer support systems. Women often face discrimination and may have limited access to healthcare and social services, exacerbating the negative impacts of their working and living environments. This highlights the urgent need for targeted interventions to address the specific challenges faced by female migrant workers in the construction sector.

The t-test examining the lack of awareness among migrant workers in the construction sector regarding labor rights and inadequate legal protections, categorized by occupation, reveals significant differences. Workers in the organized sector reported a mean score of 12.46 with a standard deviation of 2.63, while those in the unorganized sector had a mean score of 18.20 (SD=3.37). The t-value of 2.59 and a p-value of 0.02 indicate a statistically significant difference, leading to the rejection of the null hypothesis. This suggests that workers in the unorganized sector experience greater economic exploitation and hardship due to their lack of awareness about labor rights. The higher mean score among unorganized workers can be attributed to their vulnerability and limited access to information regarding legal protections. Many of these workers are often illiterate or have minimal education, which hampers their ability to understand their rights and seek redress against exploitation. Occupations in the unorganized sector, such as those working in hotels, finance, tea shops, bakeries, as bakers or drivers, and as fruit vendors or water sellers (paani), typically lack formal employment contracts and benefits, further exacerbating their precarious situation. Unorganized sector workers generally lack the support systems and resources available to those in organized employment, making them more susceptible to unfair practices by employers. This shows the urgent need for targeted educational programs and legal support initiatives to empower these workers and ensure their rights are protected, ultimately improving their economic conditions and well-being.

## RESULT AND DISCUSSION

### FACTORS DRIVING MIGRANT WORKERS TO KARNATAKA'S CONSTRUCTION SECTOR

Migrant workers are increasingly drawn to Karnataka's construction sector due to a combination of push and pull factors. Push factors include economic distress, lack of job opportunities, and inadequate living conditions in their home regions, prompting individuals to seek better prospects elsewhere. In contrast, Karnataka offers attractive pull factors such as higher wages, abundant job opportunities, and better living standards. The state's rapid urbanization

and infrastructure development create a high demand for labor, particularly in cities like Bangalore and Mysore, where construction projects are booming. The availability of social amenities, including access to healthcare and education for workers' families, enhances Karnataka's appeal. Many migrants are also motivated by the prospect of remittances, which can significantly improve their families' quality of life back home. Migrant workers constitute a significant portion of the construction workforce in Karnataka, particularly in urban centres like Bangalore, where rapid economic growth has created a high demand for labor. The analysis examines the factors prompting these workers to migrate for employment in the construction sector, focusing on the economic conditions in their places of origin, local job opportunities, and potential wage increases. Many migrant workers in Karnataka come from economically disadvantaged regions, primarily in rural areas where job opportunities are scarce. The Economic Survey of Karnataka (2016-17) highlighted that a substantial number of individuals migrate to urban areas in search of better livelihoods due to the lack of employment options in their home regions. In particular, many workers originate from areas with high poverty rates, where daily wage labor is often the only available option. A study conducted by Shamala B. (2021) indicates that the economic conditions in these regions are characterized by limited agricultural productivity and inadequate infrastructure, which force individuals to seek employment elsewhere. The construction sector in Karnataka, especially in Bangalore, offers a stark contrast to these conditions, providing a more stable income and better living standards. Karnataka's construction industry has experienced significant growth, largely driven by urbanization and infrastructure development. The state's Gross State Domestic Product (GSDP) grew by 8.2% in the fiscal year 2010-2011, with Bangalore emerging as a hub for various industries, including information technology and real estate. The growth has led to a surge in construction projects, including residential buildings, commercial complexes, and infrastructure projects such as roads and metro systems.

According to a report by SHRAM (2015), the construction sector in Bangalore alone relies on approximately 1.5 million migrant workers to meet its labor demands. The availability of jobs in construction is particularly appealing to those with limited skills and education, as it often requires less formal training compared to other sectors. The report emphasizes that the construction industry is one of the few viable employment options for these workers, making it an essential component of their migration decision.Wage differentials between Karnataka and the workers' home states serve as a significant pull factor for migration. The potential for higher earnings in Karnataka compared to their places of origin is a crucial motivator. For instance, daily wages for construction workers in rural areas may range from 200 to 300, wages in Karnataka can exceed 500 per day. This wage increase is particularly attractive for workers who aim to support their families back home through remittances. A study by Zabeer et al. (2019) found that the average daily wage for construction workers in Bangalore was significantly higher than in many other states, which incentivizes workers to migrate. The ability to earn more allows these workers to improve their living conditions and provide better educational opportunities for their children, further perpetuating the cycle of migration. Despite the economic incentives, migrant workers in Karnataka face numerous challenges. Many work in informal settings without job security or access to social benefits. According to a survey conducted by Anekal Jesuit Educational and Charitable Society (2022), a significant proportion of migrant workers reported poor living conditions, low wages, and lack of access to healthcare. The survey indicated that around 70% of households in the Anekal region, where many migrant workers reside, rely on daily wage labor, with erratic earnings that often do not meet basic needs.

The construction sector's reliance on subcontracting often leaves workers vulnerable to exploitation. The principal employers are not always held accountable for the welfare of their workers, as contractors and subcontractors may not comply with labor laws. The Inter-State Migrant Workmen (Regulation of Employment and Conditions of Service) Act (1979) is often inadequately enforced, leaving many workers without legal protections. The factors prompting migrant workers to seek employment in Karnataka's construction sector are multifaceted, encompassing economic conditions in their places of origin, the availability of local job opportunities, and the potential for wage increases. The construction industry offers a pathway to improved livelihoods, it is essential to address the challenges these workers face, including inadequate legal protections and poor living conditions. Policymakers must focus on creating robust frameworks that ensure the rights and welfare of migrant workers, facilitating their

integration into the labor market safeguarding their dignity and well-being. The approach will not only benefit the workers but also contribute to the sustainable growth of Karnataka's economy.

**MIGRANT CONSTRUCTION WORKERS IN KARNATAKA: HEALTH, HOUSING, AND ECONOMIC WELL BEING**

Migrant workers are integral to Karnataka's construction sector, significantly contributing to the state's economic growth. However, their living and working conditions often starkly contrast the benefits they provide. Health issues among migrant construction workers in Karnataka are prevalent due to inadequate access to medical facilities and poor living conditions. A study by Zabeer et al. (2019) in Bangalore highlighted that many workers suffer from chronic health problems exacerbated by stressful working environments and limited healthcare access. The average age of these workers is around 26 years, with a significant portion being male (95.2%) and engaged in physically demanding labor. The study found that workers living in substandard housing conditions reported poorer health outcomes, including higher rates of respiratory and musculoskeletal disorders. A baseline survey conducted by the Centre for Integral Rural Welfare (CIRW) in 2022 indicated that many migrant workers are malnourished and lack basic health services, contributing to their declining health status. The survey revealed that 70% of households in Anekal, a significant area for migrant labor, consist of daily wage earners with erratic incomes, making it difficult for them to afford healthcare. Housing is a significant concern for migrant workers in Karnataka. Many live in makeshift shelters or overcrowded conditions that lack basic amenities such as clean water, sanitation, and electricity.

The Economic Survey of Karnataka (2016-17) noted that the state has a high rate of interstate migration, with many workers arriving from poorer regions in search of better opportunities. However, they often end up in informal settlements that do not meet minimum living standards. According to the study by Zabeer et al. (2019), the quality of housing directly impacts the workers quality of life. Those residing in huts reported a lower quality of life compared to those in Pucca (permanent) houses, indicating that housing quality is a critical determinant of overall well-being. The lack of proper sanitation facilities further exacerbates health risks, leading to a cycle of poverty and ill health.

Economically, migrant workers in Karnataka's construction sector face numerous challenges. The average daily wage for these workers ranges from 250 to 300, which is often insufficient to meet their basic needs. A significant portion of the workforce is engaged in low-paying, informal jobs, with limited job security and benefits. The 2011 Census revealed that Karnataka had approximately 32 lakh migrants, many of whom are employed in the construction industry, yet they remain vulnerable to exploitation and fluctuating market conditions. A socio-economic study by Shamala B. (2021) highlighted that 93% of migrant workers cited the need to increase family income as their primary reason for migrating. However, the economic benefits they anticipated often do not materialize due to systemic issues such as lack of job stability and inadequate wage structures. The Inter-state Migrant Workmen (Regulation of Employment and Conditions of Service) Act, 1979, intended to protect these workers, is often poorly implemented, leaving them without necessary legal protections.The living and working conditions of migrant workers in Karnataka's construction sector are marked by significant challenges in health, housing, and economic stability. Despite their critical role in the state's development, these workers often find themselves in precarious situations, facing health risks, inadequate housing, and economic instability. Addressing these issues requires comprehensive policy interventions that focus on improving living conditions, ensuring fair wages, and providing access to healthcare. Only through such measures can the state truly harness the potential of its migrant workforce safeguarding their rights and well-being.

**POLICIES FOR IMPROVING CONDITIONS FOR MIGRANT WORKERS IN KARNATAKA**

Migrant workers play a pivotal role in Karnataka's construction sector, significantly contributing to the state's economic development. To promote better living and working conditions for migrant workers in Karnataka, There are several main policies and acts have been established, addressing various aspects of their employment and welfare. The Building and Other Construction Workers (Regulation of Employment and Conditions of Service) Act, 1996 is a significant piece of legislation that regulates the employment conditions of construction workers, ensuring their rights to safe working environments, health and safety measures, and welfare benefits. This act came into force

on March 1, 1996, and applies to establishments employing ten or more building workers, mandating provisions for their safety and welfare, as outlined in the India Code.

The Inter-State Migrant Workmen (Regulation of Employment and Conditions of Service) Act, 1979 provides a framework for the employment of inter-state migrant workers, guaranteeing their rights to safe working conditions, timely payment of wages, and access to welfare measures. This act is crucial in protecting the rights of migrant workers who often face exploitation and irregular employment . The Minimum Wages Act, 1948 establishes minimum wage standards for all workers, ensuring that migrant workers in the construction sector receive fair compensation for their labor. Despite this, many workers still report not receiving the minimum wage, highlighting a gap between legal provisions and actual practices. The Contract Labour (Regulation and Abolition) Act, 1970 regulates the employment of contract labourers, providing them with rights and protections against exploitation by contractors. This act is essential in ensuring that migrant workers, who often work under contract arrangements, are not subjected to unfair labor practices .

The Karnataka Labour Welfare Fund Act, 1965 creates a welfare fund for labourers, offering financial support for various needs, including healthcare, education, and housing. This act aims to improve the overall welfare of workers, including migrants, by providing them with essential services and support . The Maternity Benefit Act, 1961 ensures that female workers, including those in the construction sector, receive maternity benefits and job protection during pregnancy. This act is vital for supporting women workers and ensuring their rights during a critical time in their lives. The Child Labour (Prohibition and Regulation) Act, 1986 prohibits the employment of children in hazardous occupations, including construction, thereby protecting vulnerable populations from exploitation and ensuring that children have the opportunity to pursue education instead of labor . Lastly, the Equal Remuneration Act, 1976 mandates equal pay for equal work, ensuring that migrant workers receive fair wages regardless of gender. This act is crucial in addressing wage disparities and promoting gender equality in the workplace . These policies aim to create a comprehensive policy framework that addresses the multifaceted challenges faced by migrant workers in Karnataka, thereby improving their overall living and working conditions. By implementing these policies effectively, the state can better harness the potential of its migrant workforce safeguarding their rights and well-being.

## CHALLENGES AND ISSUES FACED BY MIGRANT WORKERS IN KARNATAKA'S CONSTRUCTION SECTOR.

Migrant workers in Karnataka's construction sector face numerous challenges that significantly affect their quality of life. The construction industry in Karnataka, particularly in urban areas like Bangalore and Mysore, has seen a substantial influx of migrant labor due to rapid urbanization and industrial growth. These workers often come from poorer states, seeking better economic opportunities but encounter harsh realities upon arrival.

### Living Conditions and Health Issues

One of the primary challenges faced by migrant workers is inadequate living conditions. Many reside in makeshift shelters, such as roadside tents or temporary sheds, which lack basic amenities like proper sanitation, clean water, and electricity. These living conditions contribute to a host of health problems, including malnutrition and communicable diseases. For instance, studies indicate that workers are frequently exposed to unsanitary environments, leading to illnesses exacerbated by poor housing conditions, such as cholera and respiratory issues from inhaling dust and fumes from construction materials.

### Economic Exploitation and Job Insecurity

Economic exploitation is another critical issue. Migrant workers often receive low wages for long hours of labor, with many dependent on informal contracts that offer little job security. The lack of formal recognition and registration makes them vulnerable to exploitation by contractors and sub-contractors, who may withhold wages or impose unfair working conditions. A significant portion of these workers is illiterate or has only elementary education, limiting their ability to advocate for their rights or seek better employment opportunities.

**Social Isolation and Lack of Access to Services**

Social isolation is prevalent among migrant workers, who often leave their families behind in their home states. This separation can lead to mental health issues and a lack of community support. Access to essential services such as healthcare, education for their children, and legal protections is severely limited. Many migrants are unaware of their rights and the welfare schemes available to them, which further exacerbates their vulnerability.

**Impact of Political and Economic Factors**

Political factors also play a role in the challenges faced by migrant workers. Recent events, such as the need for workers to travel back to their home states to vote, have disrupted construction activities, highlighting their precarious employment situation. The COVID-19 pandemic further intensified these challenges, with many workers losing their jobs during lockdowns and facing financial hardships without adequate government support. Migrant workers in Karnataka's construction sector confront a multitude of challenges, including poor living conditions, economic exploitation, social isolation, and limited access to essential services. Addressing these issues requires comprehensive policy interventions aimed at improving their living and working conditions, ensuring fair wages, and providing access to healthcare and education.

## FINDINGS OF THE STUDY

1. Economic hardship and limited job opportunities in their hometowns are significant factors motivating migrant workers to seek employment in Karnataka's construction sector.
2. Female migrant workers experience greater adverse effects from poor living and working conditions than their male counterparts, highlighting the need for gender-specific interventions by government and non-governmental organisations, Civil Society organisations etc.
3. Deprived living and working conditions significantly impact the health and economic well-being of migrant workers, with female workers reporting higher levels of negative health outcomes.
4. Significant and lack of awareness among migrant workers regarding their labour rights, particularly in the unorganized sectors, which contributes to their economic exploitation.
5. Many migrant workers face social isolation, which exacerbates their vulnerability and limits their access to support networks and essential services.
6. Existing policies aimed at improving the living and working conditions of migrant workers are often inadequately enforced, leading to persistent challenges in their quality of life.
7. Migrant workers frequently encounter barriers to accessing healthcare services, which negatively affect their overall health and well-being.
8. There is an urgent need for targeted educational programmes and legal support initiatives to empower migrant workers, particularly those in the unorganized sector, to ensure their rights are protected and improve their economic conditions.
9. Economic hardship and limited job prospects in their hometowns drive migrant workers to seek employment in Karnataka's construction sector. The t-test revealed a significant difference based on marital status, with unmarried respondents reporting a higher mean score (12.44, SD = 2.89) compared to married respondents (10.28, SD = 2.29). The t-value was 2.93 and the p-value was 0.05, leading to the rejection of the null hypothesis and supporting the idea that unmarried workers are more motivated by economic conditions in their hometowns.
10. Poor living and working conditions significantly impact the health and economic well-being of migrant workers. The t-test results showed that female respondents had a higher mean score (18.09, SD = 4.06) than male respondents (12.41, SD = 2.22), with a t-value of 3.19 and a p-value of 0.01. This significant difference leads to the rejection of the null hypothesis, indicating that female migrant workers face greater adverse effects from poor conditions.
11. Lack of awareness regarding labour rights contributes to economic exploitation among migrant workers. The t-test indicated that unorganized sector workers had a higher mean score (18.20, SD = 3.37) compared to organized

sector workers (12.46, SD = 2.63), with a t-value of 2.59 and a p-value of 0.02. This significant finding leads to the rejection of the null hypothesis, highlighting the need for educational initiatives to raise awareness of labor rights among unorganized sector workers.

## SUGGESTIONS

1. Need to Develop and implemented targeted economic programmes in migrants' home regions to create job opportunities, reducing the need for migration and improving local livelihoods.
2. The Government is expected to implement gender-specific policies and programmes that address the unique challenges faced by female migrant workers, including health services and safety measures in the workplace.
3. Urgent action is needed to establish and enforce rigorous health and safety regulations within the construction sector, significantly reducing workplace injuries and promoting the well-being of migrant workers.
4. There is a critical need to launch widespread educational campaigns that empower migrant workers, particularly those in the unorganized sector, by raising awareness of their labor rights and equipping them to resist exploitation.
5. Expanding access to healthcare services for migrant workers is essential, including the deployment of mobile health clinics and collaborations with local healthcare providers to guarantee timely and necessary medical care.
6. Building strong community support networks is vital to facilitate the social integration of migrant workers, providing essential resources that alleviate isolation and enhance their quality of life.
7. Strengthening the enforcement of labor laws and regulations is crucial to safeguarding migrant workers' rights, with a focus on holding employers accountable for ensuring fair treatment and the well-being of their workforce.
8. Developing affordable, safe, and adequate housing solutions for migrant workers is essential, guaranteeing access to fundamental amenities such as clean water, sanitation, and reliable electricity.
9. Investing in skill development and vocational training programs tailored to the specific needs of migrant workers is key to enhancing their employability and enabling them to secure better-paying jobs.
10. Fostering strategic partnerships between government agencies, NGOs, and community organizations is necessary to build a comprehensive support system that effectively addresses the diverse needs of migrant workers.

## CONCLUSION

Migrant workers play a crucial role in Karnataka's construction sector, driven by various economic and social factors. The primary reasons for their migration include poor economic conditions in their home states, limited local job opportunities, and the allure of higher wages in urban centres like Bangalore. Many come from economically disadvantaged regions, where poverty and lack of employment compel them to seek better prospects elsewhere. The construction industry, characterized by its demand for labor, becomes a viable option for these workers despite the inherent risks and challenges associated with such jobs. The living and working conditions of migrant workers in Karnataka are often substandard. Many reside in temporary shelters lacking basic amenities such as clean water, sanitation, and adequate ventilation. Health issues are prevalent due to exposure to hazardous materials and unsanitary living conditions, leading to a range of illnesses.

Furthermore, workers face economic instability, as their earnings are often insufficient to meet their basic needs, and they lack access to social security benefits and healthcare services. Policies aimed at improving the living and working conditions of migrant workers in Karnataka have been implemented, such as the establishment of the Karnataka Building and Other Construction Workers Welfare Board. This board facilitates access to various social protection schemes, including health services and financial assistance. NGOs like Sampark also play a vital role by raising awareness of workers' rights and providing support through resource centres. Despite these efforts, significant challenges remain. Migrant workers frequently encounter exploitation, unsafe working environments, and a lack of legal protections. Their transient status often leaves them vulnerable, with limited recourse to address

grievances. Addressing these issues requires a multi-faceted approach that includes better enforcement of labor laws, improved living conditions, and enhanced access to healthcare and education, ensuring that the rights and well-being of migrant workers are prioritized in the construction sector.

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**Table 1. Demographic Variables of respondents**

| Demographic Variable | Category | No of respondents | Percentage |
|---|---|---|---|
| **Marital Status** | Married | 25 | 37.87 |
| | Unmarried | 41 | 62.12 |
| **Gender** | Male | 32 | 48.48 |
| | Female | 34 | 51.50 |
| **Occupation** | Organized | 22 | 33.33 |
| | Unorganized | 44 | 66.66 |
| **Total respondents** | **66** | | **100%** |

Source: Survey Data

**Table 2: t- test of economic hardship and limited job prospects in their hometowns are primary factors driving migrant workers to seek employment in Karnataka's construction sector on the basis of marital status**

| Marital Status | Mean | S.D | t-value | P-value |
|---|---|---|---|---|
| Married | 10.28 | 2.29 | 2.93 | 0.05 |
| Unmarried | 12.44 | 2.89 | | |

Source: Primary data collected by the researcher
*Significant at 0.05 level

**Table 3: t- test of Poor living and working conditions among migrant workers in Karnataka's construction sector had profound impact on their health and economic well-being on the basis of gender**

| Gender | Mean | S.D | t-value | P-value |
|---|---|---|---|---|
| Male | 12.41 | 2.22 | 3.19 | 0.01 S |
| Female | 18.09 | 4.06 | | |

Source: Primary data collected by the researcher
* Level of Significance = 0.01 level

**Table 4: t- test of Lack of awareness among migrant workers in construction sector on labour rights and inadequate legal protections paved way for economic exploitation and hardship on the basis of occupation like organised and unorganised sector**

| Occupation | Mean | S.D | t-value | P-value |
|---|---|---|---|---|
| Organised | 12.46 | 2.63 | 2.59 | 0.02 S |
| Unorganised | 18.20 | 3.37 | | |

Source: Primary data collected by the researcher
*Significant at 0.05 level

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**RESEARCH ARTICLE**

# Enhanced Skin Cancer Detection through Hyperspectral Imaging and YOLO

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## ABSTRACT

Recent research has used several deep learning techniques to identify cases of skin cancer. Hyperspectral imaging (HSI) is a noninvasive optical technology that is worth investigating further since it may record wavelength information on the locations of skin cancer lesions. HSI technology improves the distinction of picture features by recording hundreds of visible and invisible narrow bands from the electromagnetic spectrum. In order to identify and categorise skin cancer forms such seborrhoeic keratosis (SK), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC), this research used the ISIC library dataset. The YOLO version 5 model was used for training after the dataset was divided into training and test sets. Five measures were used to assess the performance of the model: specificity, accuracy, sensitivity, precision and aria under curve. A confusion matrix was also used. Hyperspectral narrowband image (HSI-NBI) and RGB categorization are two models that were created and tested against each other to see which is more effective: HSI or RGB. Because these traits were more pronounced in HSI, the experimental findings showed that the HSI model recognised SCC features more accurately than the RGB model. With an accuracy of over 89%, the suggested model proved to be useful in the identification of skin cancer. This excellent accuracy highlights the clinical application potential and dependability of the model.

**Keywords:** skin cancer; hyperspectral imaging; YOLO

# INTRODUCTION

The most common and often diagnosed kind of cancer is nonmelanoma skin cancer (NMSC) [1]. Squamous cell carcinoma (SCC), seborrhoeic keratosis (SK), and basal cell carcinoma (BCC) are common types of skin cancer. The cells that make up BCC, the least aggressive NMSC, resemble epidermal basal cells [2]. Squamous cells, on the other hand, may spread abnormally and are invasive. Eighty percent of individuals with BCC, the most prevalent kind of skin cancer, generally have head and neck cancer that does not spread. The second most frequent kind of skin cancer, SCC, is characterised by aggressive tumours that are very invasive [3].The need for quick detection techniques has grown as skin cancer cases rise. Treatment for skin cancer must begin as soon as possible after discovery. Although physicians often do traditional biopsy, it is a painful, difficult, and time-consuming procedure that involves taking a sample from a suspected cancerous location and analysing it medically [4]. Advances in artificial intelligence (AI) have resulted in the creation of many computer-aided detection (CAD) models for different types of cancer [5-7]. For instance, Haenssle et al. [8] used the InceptionV4 model to diagnose skin cancer and discovered that although it performed worse in specificity by 9%, it performed better in sensitivity, outperforming 58 dermatologists by 8%. Using the Asan training dataset, Han et al. [9] used ResNet-152 and obtained findings that were similar to those of sixteen dermatologists. Using a deep learning technique on 6000 photos, Fujisawa et al. [10] were able to identify benign and malignant cases with 76% accuracy. Even if CAD and biosensors provide affordable alternatives for early diagnosis, issues such nanoparticles' environmental adaptation still exist. Red, green, and blue (RGB) imaging-based conventional CAD models have achieved saturation. Hyperspectral imaging (HSI), on the other hand, offers a viable substitute that could get beyond the drawbacks of conventional techniques.

Rather of restricting each pixel to one of the three basic colours, hyperspectral imaging (HSI) is a state-of-the-art method that analyses a wide variety of wavelengths [11-12]. Applications for High Sensitivity Imaging (HSI) include agriculture, cancer detection, military, air pollution detection, dentistry imaging, environmental monitoring, satellite photography, forestry monitoring, food security, natural resource surveying, vegetation observation, and geological mapping [13-17]. A number of research have also looked at the use of HSI to the diagnosis of skin cancer. Leon et al. [18] created a system to automatically recognise and categorise pigmented skin lesions (PSL) by combining supervised and unsupervised learning approaches. In order to determine the best spectral wavelengths for differentiating between normal and malignant skin, Courtenay et al. [19] developed a specialised platform that combined a visible-near infra-red (VNIR) hyperspectral imaging sensor. Courtenay et al. used HSI in a different investigation to identify spectral changes between specimens of normal and malignant cutaneous tissue. Nevertheless, existing techniques often depend on costly and intricate HSI imaging sensors or cameras [20].

This research suggests a quick skin cancer diagnosis technique that combines HSI technology with the YOLO version 5 model in order to overcome these difficulties. The five variables used to assess the performance of the created model were sensitivity (SEN), accuracy (ACC), specificity (SPEC), precision (PREC) and aria under curve (AUC).

# METHODS

**Data Preprocessing**

Three skin-related conditions are the subject of this study: seborrhoeic keratosis (SK), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC). The dataset includes 168, 90, and 126 photos for the same categories in the validation set and 654, 336, and 480 images for BCC, SCC, and SK, respectively, in the training set. Total training and validation pictures utilised were 1470 and 384, respectively [5]. Figure 1 illustrates the division of the work into three key components: database development, picture preprocessing, and assessment of the YOLOv5 model training outcomes. This study made use of the ISIC dataset. Figures 2–4 show instances of seborrhoeic keratosis, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC), in that order.

During preprocessing, 640 × 640 pixels were the new size for each picture. The photos were annotated using LabelImg software, which created XML files. After that, the files were converted to TXT format and used as training data for the YOLOv5 model. We used a spectrum conversion technique to convert the annotated picture files into hyperspectral narrow-band images (NBI) prior to training. As shown in Figure 5, the research first compared the normalised reflection spectrums of the lesion sites to analyse the three categories of skin cancer situations. We examined wavelength bands with notable variations in the intensity of reflected light. The 380–780 nm region of the spectrum was transformed into an HSI with a spectral resolution of 1 nm. Principal component analysis (PCA) was used to reduce the burden of this dimensional data's high processing and storage requirements. PCA reduced the dimensionality of the picture, extracted significant feature portions, and generated new component information by linearly transforming the data into a new space. The outcome was a projection of the data with decorrelated spectral information onto a low-dimensional space.

RGB and HSI photos were used to construct two datasets. Eighty percent of the data was put aside for testing and twenty percent was set aside for training. This separation came about after the amplification of images for both datasets. The Python 3.9.12 platform was used to write the programme for the PyTorch deep learning framework, which was applied to the Windows 10 operating system.

**YOLOv5 Model**

For real-time performance, this research specifically chose YOLOv5 because of its faster detection speed than other models, such as SSD and RetinaNet. The three essential components of YOLOv5 are the head terminals, neck, and backbone (Figure 6).

**Backbone**

Focus, CONV-BN-Leaky ReLU (CBL), cross stage partial (CSP), and spatial pyramid pooling (SPP) are some of the models that make up the convolutional neural network (CNN) architecture. By slicing the input picture, the focus model produces image features, collects many fine-grained images, boosts forward and back propagation speed, and uses less CUDA memory and fewer layers. The approach involves dividing an input picture of 640×640×3 into four smaller images of 320×320×3. These images are then combined and subjected to convolution layers to create an output image of 320×320×32. This reduces the image size and speeds up training [21].

**Neck**

CBL, Upsample, CSP2_X, and more models make up the neck. It acts as a sequence of feature aggregation layers that creates feature pyramid (FPN) and path aggregation (PAN) networks by combining features from images. To improve image feature extraction and minimise model size, YOLOv5 adds the CSP2_X structure while keeping the CSP1_X structure from YOLOv4's CSPDarknet-53.

**Head**

The head maximises the bounding box forecasts' accuracy by using GIoU Loss as the loss function. For the suggested skin cancer classification work, YOLOv5 is the best option because of its structure, which guarantees effective and precise detection.

## RESULTS AND DISCUSSION

After 300 rounds of training the loss function, the value curves of the training and validation sets were calculated, with a batch size of 16. Out of the 384 test photos, the RGB model properly predicted 333 images, whereas the HSI model correctly recognised 322 images. The effectiveness of the suggested technique was assessed using pre-established metrics such as Sensitivity (Sen), Accuracy (Acc), Specificity (Spe), Jaccard index value (Jac), and Dice coefficient score (Dic). The following formulae are employed:

$$SEN = \frac{TP}{TP + FN}$$
$$SPEC = \frac{TN}{TN + FP}$$
$$ACC = \frac{TP + TN}{TP + TN + FN + FP}$$
$$PREC = \frac{TP}{TP + FP}$$

Figure 7 and Figure 8 shows the confusion matrix of YOLOv5-based suggested model for detection, respectively. These data assist visualise the incidences of skin cancer that were properly and mistakenly predicted for both the RGB and HSI models, as well as the model's performance in identifying the different forms of skin cancer. Table 1, Table 2 and Table 3 shows the classifier comparisonof BCC, SCC and SK datasets. The HSI models' accuracygreater than 89% demonstrated their resemblance. When compared to the original picture category SCC, the more noticeable SCC category in the HSI model shows a more notable improvement. This result showed how very comprehensive the model is when it comes to learning lesion characteristics. Because of the very limited number of photos utilised, it is evident that the accuracy rates of the RGB model and the HSI model are equal. All the same, this work demonstrates the possibility for accurate skin cancer detection and classification using HSI-based conversion algorithms that can transform RGB photos into HSI images. This study greatly increased the recall rate and specificity of the SCC category as well as the specificity (true negative rate) for the SK category, even if the majority of the findings were identical.

It is clear from the results and discussion section that our suggested approach—which combines hyperspectral imaging (HSI) with YOLOv5—performs better than other well-known classifiers in the identification of skin cancer. The thorough investigation revealed that while the HSI models' accuracy was significantly improved, especially for the SK category. The RGB model provided a better degree of completeness in learning lesion information, while the HSI-based conversion algorithms demonstrated efficacy in skin cancer detection and classification. This study opens the door for more precise and trustworthy diagnostic instruments by demonstrating the ability of conventional RGB imaging technology to improve the performance of skin cancer detection models. The enhanced specificity for certain categories confirm that the suggested approach is more effective than hyperspectral classifiers and may lead to improved diagnostic results.

## CONCLUSIONS

YOLOv5 is mainly used to classify skin cancer pictures into three categories: SCC, BCC, and SK based on the CNN architecture. To generate a confusion matrix and determine the accuracy, specificity, sensitivity and precision values—which are used as classification indicators—lesion categories, such as RGB and hyperspectral datasets, are used. The outcomes of the experiment demonstrated that the SCC category in the HSI model is capable of picking up distinguishing traits. The original RGB picture is not as good at detection as the HSI classification model. YOLOv5 is easier to use than its predecessors since it has the ability to automatically scale the supplied picture to the desired size. At a quicker pace, the comparatively compact model structure attains an accuracy comparable to the preceding generation. The experiment's following designs may use the gain to increase accuracy in other studies.

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**Table 1: Classifier comparison for BCC**

| Classifier | Method | ACC | SEN | SPEC | PREC | AUC |
|---|---|---|---|---|---|---|
| **YOLO v5** | **Proposed Method** | **86.72** | **90.53** | **83.72** | **81.38** | **87.13** |
| TREE | CT | 82.03 | 84.62 | 80.00 | 76.88 | 82.31 |
| | ST | 78.39 | 83.43 | 74.42 | 71.94 | 78.93 |
| SVM | LSVM | 81.25 | 82.25 | 80.47 | 76.80 | 81.36 |
| | CSVM | 82.29 | 81.66 | 82.79 | 78.86 | 82.22 |
| | QSVM | 80.73 | 82.25 | 79.53 | 75.96 | 80.89 |
| | MGSVM | 81.77 | 82.25 | 81.40 | 77.65 | 81.82 |
| KNN | FKNN | 82.55 | 84.02 | 81.40 | 78.02 | 82.71 |
| | MKNN | 81.25 | 83.43 | 79.53 | 76.22 | 81.48 |
| | Cosine | 81.25 | 84.02 | 79.07 | 75.94 | 81.55 |
| | Cubic | 79.69 | 81.07 | 78.60 | 74.86 | 79.83 |
| | WKNN | 77.86 | 79.29 | 76.74 | 72.83 | 78.02 |

**Table 2: Classifier comparison for SCC**

| Classifier | Method | ACC | SEN | SPEC | PREC | AUC |
|---|---|---|---|---|---|---|
| **YOLO v5** | **Proposed Method** | **88.80** | **88.89** | **88.78** | **70.80** | **88.83** |
| TREE | CT | 83.59 | 77.78 | 85.37 | 61.95 | 81.58 |
| | ST | 83.59 | 84.44 | 83.33 | 60.80 | 83.89 |
| SVM | LSVM | 84.90 | 82.22 | 85.71 | 63.79 | 83.97 |
| | CSVM | 85.68 | 83.33 | 86.39 | 65.22 | 84.86 |
| | QSVM | 84.64 | 82.22 | 85.37 | 63.25 | 83.80 |
| | MGSVM | 85.16 | 83.33 | 85.71 | 64.10 | 84.52 |
| KNN | FKNN | 85.94 | 82.22 | 87.07 | 66.07 | 84.65 |
| | MKNN | 80.73 | 76.67 | 81.97 | 56.56 | 79.32 |
| | Cosine | 79.69 | 67.78 | 83.33 | 55.45 | 75.56 |
| | Cubic | 77.86 | 64.44 | 81.97 | 52.25 | 73.21 |
| | WKNN | 78.91 | 65.56 | 82.99 | 54.13 | 74.27 |

**Table 3: Classifier comparison for SK**

| Classifier | Method | ACC | SEN | SPEC | PREC | AUC |
|---|---|---|---|---|---|---|
| **YOLO v5** | **Proposed Method** | **89.06** | **88.89** | **89.15** | **80.00** | **89.02** |
| TREE | CT | 84.64 | 79.37 | 87.21 | 75.19 | 83.29 |
| | ST | 84.64 | 78.57 | 87.60 | 75.57 | 83.08 |
| SVM | LSVM | 83.07 | 77.78 | 85.66 | 72.59 | 81.72 |
| | CSVM | 82.29 | 76.98 | 84.88 | 71.32 | 80.93 |
| | QSVM | 82.81 | 77.78 | 85.27 | 72.06 | 81.52 |
| | MGSVM | 81.25 | 74.60 | 84.50 | 70.15 | 79.55 |
| KNN | FKNN | 82.55 | 76.19 | 85.66 | 72.18 | 80.92 |
| | MKNN | 82.55 | 76.98 | 85.27 | 71.85 | 81.13 |
| | Cosine | 81.25 | 75.40 | 84.11 | 69.85 | 79.75 |
| | Cubic | 79.95 | 76.19 | 81.78 | 67.13 | 78.99 |
| | WKNN | 78.39 | 79.37 | 77.91 | 63.69 | 78.64 |

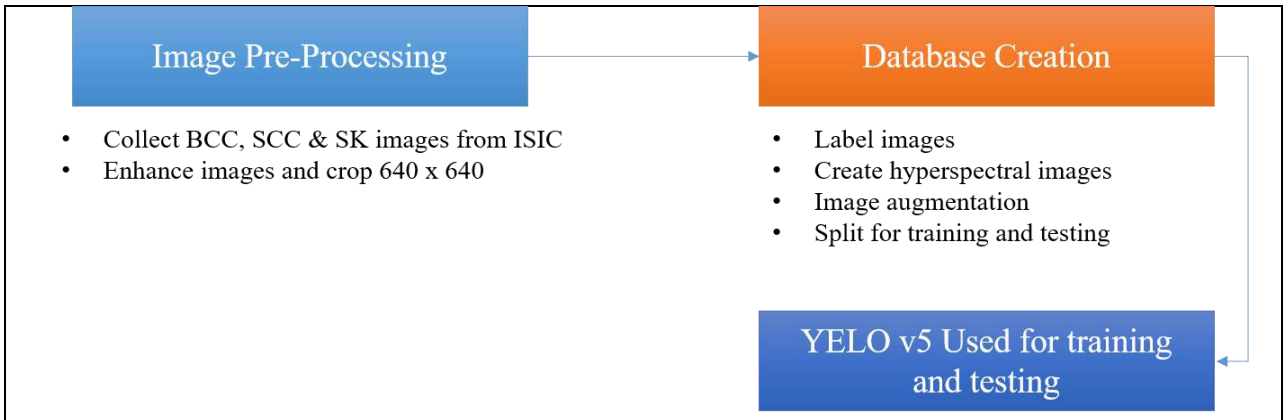


**Figure 1: Overall flow chart for the experiment**

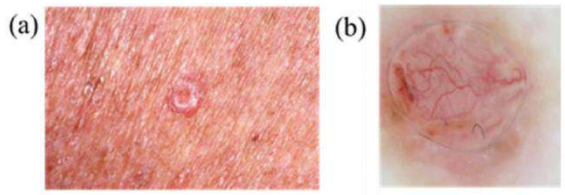


**Figure 2:BCC a) glowing projections around the epidermis b) larger lesions that frequently bleed.**

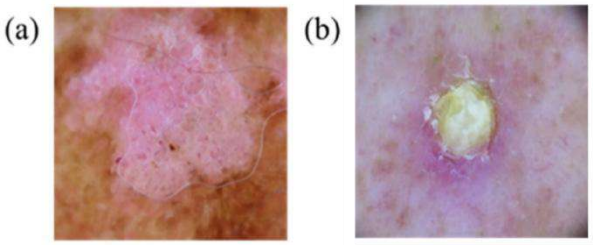


**Figure 3:SCC a) red squamous plaques b) nodules**

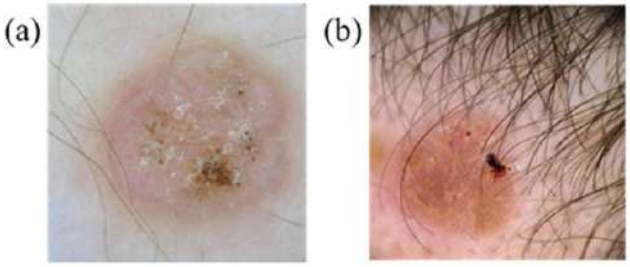


**Figure 4:SK a) as a result of mutant epidermal keratinocyte hyperplasia b) keratinocyte mass in black colour**

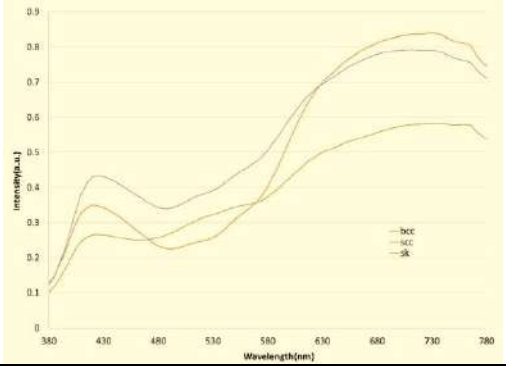


**Figure 5:BCC, SCC, and SK normalised reflection spectra. BCC is represented by the orange line, SCC by the blue line, and SK by the green line.**

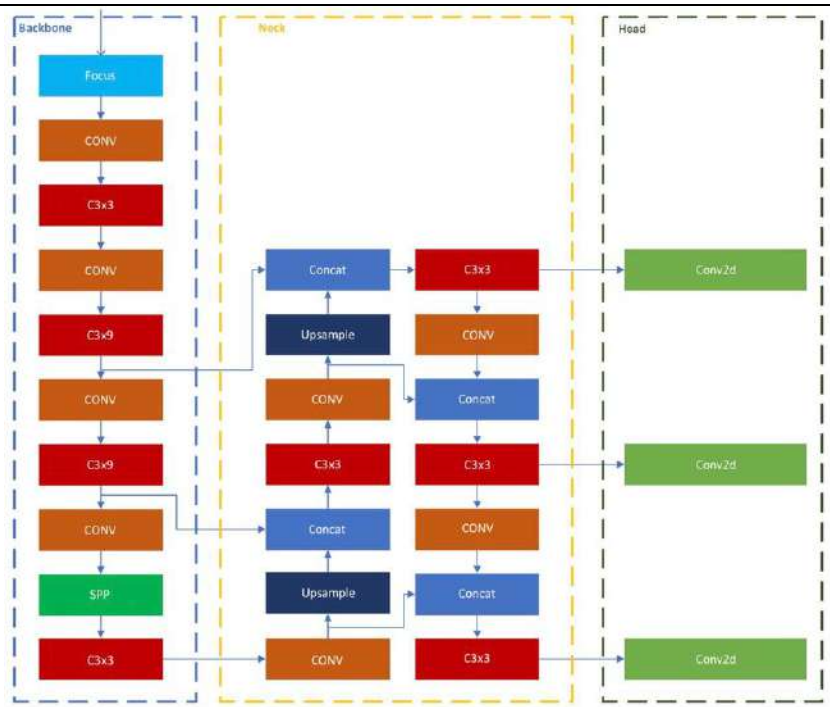


**Figure 6:Yolov5's network architecture.**

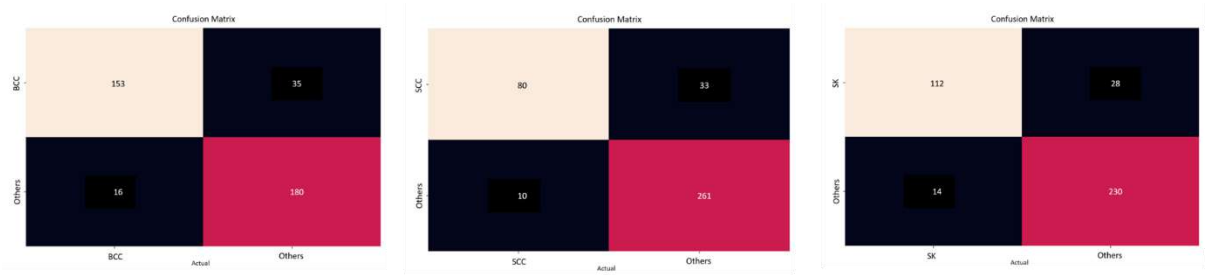


**Figure 7:confusion matrix of YOLOv5 for RGB Model**

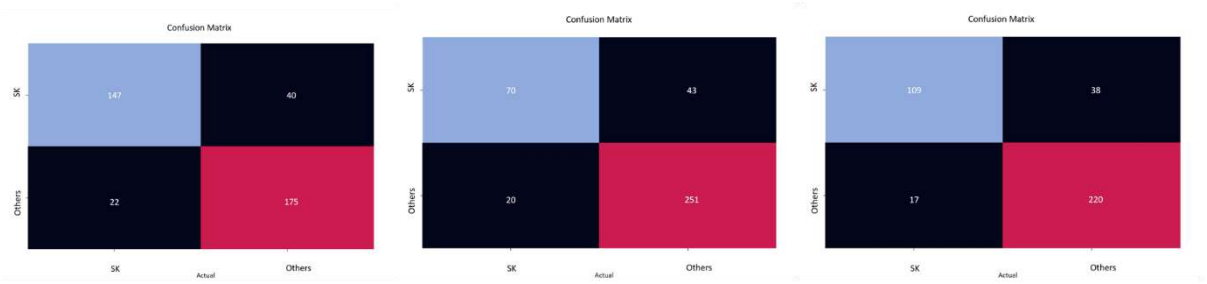


**Figure 8:confusion matrix of YOLOv5 for HSI Model**

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**RESEARCH ARTICLE**

# Qualitative Analysis and Antipyretic Validation of Siddha Formulation Pancha Moola Kudineer Chooranam

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**ABSTRACT**

*Pancha Moola KudineerChooranam*(PMKC) is a Siddha herbal formulation. This study is focussed on the qualitative analysis using HPTLC, FTIR and UV - Visible spectroscopy. Further the antipyretic activity was determined by protein denaturation assay for PMKC formulation. PMKC formulation was prepared as a decoction and it was analysed for its protein denaturation activity with Diclofenac sodium as standard. The decoction was determined for its phytoconstituents through HPTLC and FTIR analysis. Also, the UV- Vis spectroscopy was used to detect the interferenceof extractions solvent**.** PMKC at 500µg/ml concentration significantly reduced 36.61± 2.34 % of protein. HPTLC analysis revealed 8 prominent components. The UV -Vis spectroscopy obtained 254 nm, 274 nm and 361 nm as it's characteristic peaks.FTIR determined the functional groups present in the formulation.**Conclusion:** The qualitative analysis characterized the drug formulation of PMKC and determined to have 8 versatile components. The antipyretic activity was found to be higher at 500 µg/ml.

**Keywords**: Herbal formulation, PMKC, HPTLC, Antipyretic activity, FTIR.

## INTRODUCTION

Siddha medicine predominantly practiced in southern part of India is a vast collection of literature written by various saints called *siddhars* in various period of time. These *siddhars* have explored about 4448 diseases including internal and external medication for those diseases. There are 32 types of internal medication written by the saints and almost

all the parts of the plant from the roots to its secretions i.e. resins, gums, animals and its byproducts, metals and minerals have been used in the preparation of the siddha formulation after proper detoxification procedures. One such internal medicine preparation is called *kudineer* [1] (decoction) which is prepared by boiling dried coarse powder of raw drugs or fresh leaves with required amount of water until it reaches its required concentration. The often-used concentration of the decoction is one by eight or one by four of the water added. The shelf life of decoction is 3 hours. This study is aimed to scientifically prove the anti-pyretic activity of *Pancha moola kudineer Chooranam [2].*It is one of the formulations mentioned in the Siddha Classical literature *"Therayarmahakarisal"* written by the saint Therayar. He has explained that the decoction made from this siddha formulation can be used in the treatment of *suram*(fever)hence the anti-pyretic activity was carried out. The word *Pancha* means five and *Moolam* means root hence the formulation is comprised of five siddha herbal roots namely *Glycyrrzhiza glabra, Phyllanthus emblica, Chukrasiatabularis, Terminalia chebula* and *Terminalia bellirica.*

According to siddha literature the term *suram*[3]is considered as increase in the body temperatue and the main cause of fever is *seetham* or*aamam*in the gastro intestinal tract of human body, which means derangement in the normal physiological functions of the gastro intestinal tract caused by various factors like constipation, food poisoning, toxicity, increased physical activity, sleeplessness, excessive eating, having a heavy meal in excessive hunger, drinking cold water, etc. The signs and symptoms are lack of interest in food, heaviness of body, dyspepsia, loss of taste, giddiness, myalgia, chills, insomnia, xerostomia, dislike towards the sweet, sour and salt tastes, increased sweating, etc.There are 64 types of *suram*among which 52 are caused by natural factors and 12 types are caused by foreign factors.

## MATERIALS AND METHODS

***Pancha moola kudineerchooranam* constituents and preparation**

The raw drugs used in the preparation of the decoction was collected and authenticated by the botanist in National Institute of Siddha, Tambaram Sanatorium. The raw drugs were dried, detoxified and made into coarse powder. The coarse powder of PMKC was used in the preparation of decoction.

**Protein denaturation assay [5] [6]**

The protein denaturation assay of the sample PMKC was studied by albumin denaturation methodology. The assay was carried out by mixing 5%aqueous solution of bovine serum albumin with thesample drug PMKC in five concentrations ranging from 100 to 500µg/ ml. 100µg/ ml of diclofenac sodium was used as the standard drug. 1N hydrochloric acid was used to adjust the pH. The incubation was done for 20 minutes at 37 °C and heated for 3 minutes at 57 °C, phosphate buffer was used and the turbidity was measured using spectrophotometer at 660nm. Distilled water was used as control. The assessment of protection from denaturation was done using the below mentioned formula.

Protein inhibition percentage: $\left[\frac{A(control\ )-A(sample\ )}{A(control\ )}\right] \times 100$

**High Performance Thin Layer Chromatography analysis [7].**

Silica gel 60F254, 7X6 cm was used in the thin layer chromatography subjected in one dimensional ascending method for the sample PMKC. Micro pipette was used to spot the PMKC for TLC. 5 tracks of PMKC were placed with 10 micro litres at a distance of 1 cm. The sample PMKC was analysed at 254 nm and 365 nm of UV light, using twin trough chamber after it was dried. HPTLC is an innovative technique derived from TLC, since pre coated HPTLC graded plates are used, this helps in obtaining remarkable output in both qualitative and quantitative aspect. This method is very efficient in assessing the phytoconstituents of herbal raw drug in one step. The chromatographic fingerprinting is helpful in identifying the phytoconstituents and its purity.

**Chromatogram Development**

CAMAG Twin Trough chambers was used in this HPTLC technique. According to the adsorption capability of herbal formulation, elution of the sample was carried out.After elution the plates are removed from chamber and dried.

**Scanning**

The Rf values were noted using the chromatographic fingerprinting of the phytocomponents. CAMAG software was used for the analysis of the data and the HPTLC plates were scanned at 366nm UV.

**Fourier Transform Infra-Red spectrum analysis [8]**

The sample was processed using Bruker Alpha-E by ATR module (attenuated total reflectance). It was ample positioned on the Crystal platform with perfect alignment of keeping anvil in upright position to assure the proper angle with the crystal prior to startof the IR radiation exposure. Spectra measurement was achieved with desired wavelengthand the corresponding observational peaks/ waves were recorded. The wavenumber wassubjected to further interpretation. Software used for the analysis is OPUS version 7 for functional group analysis. Signal detection processed through DTGS detector. Baselinecorrection adjusted as per the requirement.

**UV – Visible spectrum analysis [9]**

The sample *Pancha Moola Kudineer Chooranam* was analysed using UV visible Jasco V-750 model with the wavelength ranging of 200nm to 800nm.

**Statistical analysis**

Mean and standard deviation were used to express the results. The difference between experimental groups were analysed using ANOVA and Dunnets test

## RESULTS

**Protein denaturation assay**

The result from this analysis shows the effective heat induced albumin denaturation. by the sample drug PMKC.The maximum percentage protein inhibition found at a concentration of 500µg/ml was approximately 36.61±2.34% compared to diclofenac sodium, the standard antibiotic at 100µg/ml. The value is 91.46±1.33%. An increase in protein inhibition was observed with increasing PMKC concentration.

**High Performance Thin Layer Chromatography Analysis**

HPTLC fingerprinting was performed on thePMKC and eight major peaks were observed, confirming the presence of eight beneficial plants. The Rf values obtained variesbetween 0.08 and 0.67.

**Fourier Transform Infra-Red spectrum analysis**

FT-IR spectroscopy technique deciphered the structural information through vibration of their constituentsatoms. The functional groups were identified through their corresponding wavelength peaks that were tabulated in table 3. The peak at 3424 $cm^{-1}$ correlate to hydroxyl stretching (-OH) and ammonia group ($NH_2$). The 2914 $cm^{-1}$ correlate to CH stretching, 2811 and 2231 $cm^{-1}$ is in accord to NH stretching, 2145 $cm^{-1}$ corresponds to CN and N=C=S stretching, 1539 $cm^{-1}$ corresponds to NH deformation, 1512 $cm^{-1}$ peak confirms the presence of amide group, 1426 corresponds to CH deformation, 1322 and 1016 $cm^{-1}$ to COstretching. The peak at 940 $cm^{-1}$ is in accordance with aliphatic COOH, 832 $cm^{-1}$denotes aromatics OH deformation, 514 corresponds to COC deformation, whereas 452 corresponds to aromatic C-OH in plane bending vibration.

**UV – Visible spectrum analysis**

The absorption peak at 254.5 nm, 274 nm and 361 nm are the characteristic peak obtained for the given sample ' *Pancha Moola KudineerChooranam*.

## DISCUSSION

The temperature of human body is maintained by the thermoregulatory centre of the hypothalamus, any kind of inflammation in the body may lead to increase in the body temperature which is termed as fever so, the protein denaturation assay which is used to analyse the anti-inflammatory activity has been used as a tool to show that decrease in the inflammation in human body will lead to decrease in body temperature i.e., the probability to attain the normal body temperature is high [10][11]. The decoction made of PMKC showed Maximum percentage inhibition at 500µg/ml so this siddha herbal formulation can be used as an anti-pyretic drug. The HPTLC analysis gives the insight of the phytoconstituents present in the siddha herbal formulation PMKC. From the results it is evident that there are 8 peaks visualised under 366nm. The Rf valuevaries from 0.08 to 0.67. the highest concentration of the phytoconstituent was found to be 29.60% corresponding to the Rf value 0.14 and the eight peaks represents eight phytoconstituents present in the PMKC which may have the therapeutic value. The FTIR analysis showed the presence of alkyl group, hydroxyl group, amide group, aliphatic carboxylic acid group, aromatic hydrocarbon group and various other chemical bonds present in the phytoconstituents of PMKC. The qualitative analysis of this herbal formulation was done using UV-Vis spectroscopy technique, that provides information about residual solvent escaped into the medicine formulation through extraction process. The characteristic peak obtained in this study revealed the absence of any such extraction component and the medicinal formulation is completely of higher standard.In conclusion, the research findings of the present study have qualitatively analysed the herbal medicine formulation *Pancha Moola KudineerChooranam*. The protein denaturation assay indicated theantipyretic activity of the medicine that could be effective for treatment of suram,additionally, further *invivo* and *invitro* research should be carried out to determine the complete therapeutic value of this siddha herbal formulation.

**Conflict of interest**

The authors declare no conflict of interest.

## ACKNOWLEDGEMENT

The authors are thankful to the Sidha Central Research Institute, Arumbakkam for spectroscopy analysis. We thank the Noble research solution for HPTLC and FTIR analysis.

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**Table 1. The raw drugs used in PMKC are**

| Botanical name | Tamil name | Family | Chemical constituents [IV] |
|---|---|---|---|
| ***Glycyrrhiza glabra*** | *Adhimadhuram* | Fabaceae | • Glycyrrhizin,<br>• Glycyrrheticacid<br>• Glabridin<br>• Liquiritin<br>• Triterpene<br>• Saponin |
| ***Chukrasiatabularis*** | *Aayilpatta* | Meliaceae | • Sitosterol<br>• Quercetin<br>• 7-Dimethoxycoumarin Scopoletin,<br>• Cedrelone, |
| ***Terminalia chebula*** | *Kadukkai* | Combretaceae | • Gallic acid<br>• Chebulagicacid<br>• Punicalagin<br>• Chebulanin<br>• Corilagin<br>• Neochebulinicacid<br>• Ellagic acid<br>• Chebulinic acid |
| ***Terminalia bellirica*** | *Thandrikkai* | Combretaceae | • Tannin<br>• Pseudotannins<br>• Gallic acid<br>• Chebulicacid<br>• Chebulagicacid<br>• Ellagitannins<br>• Corilagin<br>• Ellagic acid |
| ***Phyllanthus emblica*** | *Nelliakai* | Euphorbiaceae | • Gallic acid<br>• Ellagic acid<br>• Chebulinic acid<br>• Chebulagicacid |

| | | | |
|---|---|---|---|
| | | | • Emblicanin<br>• Citric acid |

**Table 2. Protein inhibition results of PMKC and standard drug**

| Concentration (µg/ml) | Protein denaturation inhibition in percentage |
|---|---|
| PMKC 100 | 9.141±5.11 |
| PMKC 200 | 14.43±3.70 |
| PMKC 300 | 20.81±1.90 |
| PMKC 400 | 30.23±3.69 |
| PMKC 500 | 36.61±2.34 |
| Diclofenac sodium(100µg) | 91.46±1.33 |

**Table 3. HPTLC Peak table**

| Peak | Start Rf | Start height | Max Rf | Max height | Max % | End Rf | End height | Area | Area% |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 7.5 | 0.06 | 93.8 | 21.58 | 0.08 | 78.8 | 2047.4 | 27.22 |
| 2 | 0.08 | 80.1 | 0.12 | 128.6 | 29.60 | 0.14 | 65.3 | 2410.4 | 32.05 |
| 3 | 0.14 | 79.7 | 0.15 | 80.8 | 18.59 | 0.21 | 0.0 | 875.4 | 11.64 |
| 4 | 0.22 | 0.7 | 0.23 | 16.0 | 3.67 | 0.25 | 0.7 | 113.6 | 1.51 |
| 5 | 0.25 | 0.5 | 0.28 | 46.2 | 10.63 | 0.31 | 0.1 | 639.3 | 8.50 |
| 6 | 0.39 | 0.2 | 0.43 | 27.4 | 6.30 | 0.47 | 13.5 | 568.2 | 7.56 |
| 7 | 0.48 | 12.9 | 0.51 | 26.3 | 6.05 | 0.58 | 0.6 | 597.7 | 7.95 |
| 8 | 0.67 | 0.3 | 0.71 | 15.5 | 3.57 | 0.75 | 2.4 | 268.2 | 3.57 |

**Table 4. FT-IR spectrum peak table**

| Major Absorption Peak | Range in cm-1 | Functional Group |
|---|---|---|
| 1. | 3424 | May be due to NH2 stretching vibration and O-H stretching vibration, broad |
| 2. | 2914 | May be due to Broad O-H stretching and C-H vibrations |
| 3. | 2811 | May be due to N-H stretching vibration |
| 4. | 2145 | May be due to CN and N=C=S stretching vibration |
| 5. | 2231 | May be due to O-H and N-H stretching vibration |
| 6. | 1539 | May be due to N-H deformation |
| 7. | 1512 | May be due to existence of amide group |
| 8. | 1426 | May be due to C-H deformation |
| 9. | 1322 | May be due to C-O stretching and O-H deformations |
| 10. | 1016 | May be due to C-O stretching |
| 11. | 940 | May be due to aliphatic COOH |
| 12. | 832 | May be due to aromatic O-H deformation |
| 13. | 514 | May be due to C-O-C deformation |
| 14. | 486 | May be due to aromatic C-C skeleton vibration |
| 15. | 452 | May be due to aromatic C-OH in-plane bending vibration |

**Fig 1. TLC Visualisation of PMKC at 360 nm**

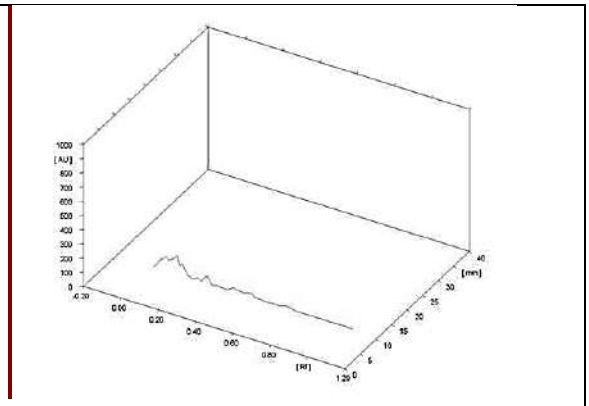
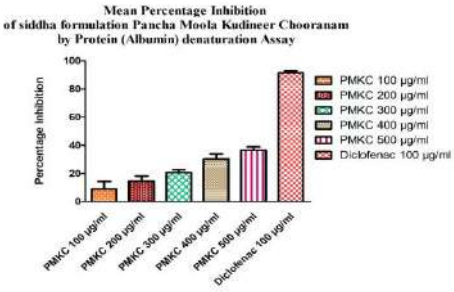


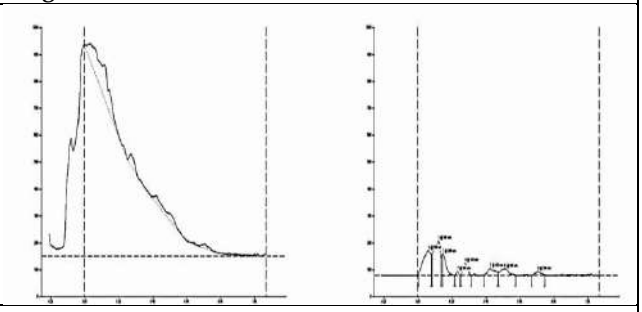
**Fig 2. 3D chromatogram**

***Fig 3.PanchaMoola Kudineer Chooranam***

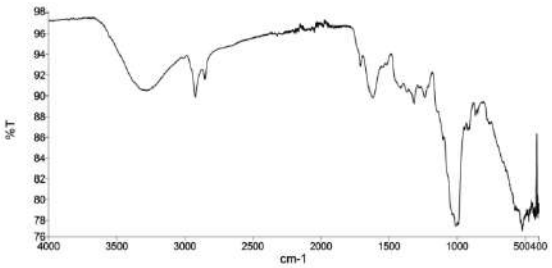
**Fig 4. Decoction of*PanchaMoolaKudineer Chooranam***




**Graph 1.Protein inhibition of PMKC and Diclofenac sodium**



**Graph 2.1, 2.2 HPTLC fingerprinting of PMKC**




**Graph 3. FT-IR spectrum of Sample PMKC**

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**RESEARCH ARTICLE**

# Allelopathic Proclivities of Tree Leaf Extracts on Seed Germination and Growth of *Aracheis hypogea* L. and *Vigna mungo* (L.) Hepper

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**ABSTRACT**

The Pot experiment was conducted in the present study to evaluate the allelopathic potential of *C. siamea* tree leaves against *A. hypogea* and *V. mungo*. Grinded leaves of *C. siamea*were soaked in tap water for 5 hours at room temperature. The different concentrations of tree species were 10, 20, 30, 40 and 50g/L. A completely randomized design having three repeats was used. Ten seeds of each species were sown in pots and then irrigated with the respective extracts soon after sowing. Results showed that germination percentage and seedling length, pigments, phenol, soluble sugar and catalase of both species were significantly affected by different concentrations of *C. siamea*. The extracts showed that stimulatory effect on germination of both test species of ground nut and black gram. The Maximum germination percentage (100%) was recorded at 10g/L extract treatment of *C. siamea.* Similarly, the maximum plant height was also observed at 41.66 cm in ground nut and 40.2 cm in black gram respectively. Low concentration of *C. siamea* extract treatment proved stimulatory effects in seedlings as compared to higher concentrations. Higher concentrations treatment showed a negative effect on the species tested. The biochemical contents were also reduced at 50 g/L extract treatment over control. Hence it can be concluded from the results that allelopathy effects of trees can be concentration dependent. The stimulatory and inhibitory effects are also different from different species and concentration also affect the crops. Presence of different allelochemicals, *C. siamea* extract can be used as a viable weed management technique in the future.Between the two test crops more inhibition was observed in black gram than in ground nut.

**Keywords:** Allelopathy, allelochemicals, germination, control, inhibition, effects, species.

## INTRODUCTION

Allelopathy is a widespread biological phenomena in which one organism creates biochemicals that affect the growth, survival, development, and reproduction of others. These biochemicals, known as allelochemicals, can have

positive or negative impacts on target species. Plant allelopathy is a mode of interaction between receptor and donor plants that can have either positive or negative effects. To maintain sustainable agricultural development, cultivation strategies that take use of allelopathic plants' stimulatory/inhibitory influence must be used to govern plant growth and development while avoiding allelopathic autotoxicity. Allelochemicals may be employed as growth regulators, herbicides, insecticides, and antimicrobial crop protection treatments. The "inhibitory" chemical is released into the soil environment where it affects the development and growth of neighbouring plants Allelopathic chemicals can be present in any part of the plant. They can be found in leaves, flowers, roots, fruits, or stems. They can also be found in the surrounding soil These toxins affect target species in many different ways. The toxic chemicals may inhibit shoot/root growth, they may inhibit nutrient uptake, or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of a nutrient[1].

Not all plants have allelopathic tendencies. Some, though they exhibit these tendencies, may be displaying aggressive competition of a non-chemical form Much of the controversy surrounding allelopathy is in trying to distinguish the type of competition being displayed. In general, if it is chemical, then the plant is considered allelopathic. There have been some recent links to plant allelotoxins directed at animals, but data is scarce. Allelopathy is emerging as a new discipline in agricultural sciences because it is hoped that the use of this science will be proved environment friendly, cost effective and cheaper. Numerous scientists have argued that a detailed study of allelopathy can reduce the reliance on herbicides.Sidhu and Hans [2] found that when the concentration of Eucalyptus extracts increased, plant growth reduced. Phlomina and Srivasuki [3] reported that leaf leachates from five multipurpose tree species (Eucalyptus camaldulensis, Acacia nilotica, Derris indica, Cassia siamea, and Sesbania grandiflora) exhibited variable degrees of inhibitory and stimulating effects on germination percentage. May and Ash [4] concluded that Eucalyptus stunted the growth of several species. Hunshal *et al*. [5] conducted allelopathic investigations and examined the chemical composition of tree species. Cheema *et al*. [6] studied for the commercial use of sorghum water extracts for weed control in wheat.

Agroforestry is a land use system, that integrates trees, cropsand/or animals in a scientifically sound way,practically feasible, ecologically desirable and socially acceptable by the farmers[7]. In the present marketoriented world the dimensions of agroforestry have changed from subsistence to commercial and Eco-friendly with a rider of maintaining a balance betweenecology and economy. Agroforestry cropping systems occupy an intermediate position, between natural and agro ecosystems, on a scale measuring domestication of the production environment. This is a result of blending agriculture with forestry. These systems should be designed to mimic at least some processes of natural forest ecosystems to obtain benefits that must otherwise accrue to the system externally. Woodmansee [8] compared natural with agricultural ecosystems, and concluded that natural ecosystems have developed several mechanisms to sustain productivity that could be exploited in properly designed agricultural systems.

Allelochemicals have a variety of effects, including decreased plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, osmotic potential, dry matter production, leaf area expansion, stomatal aperture size, stomatal diffusive conductance, and photosynthesis[9]. Allelochemicals are mostly secondary metabolites that are released into the environment via natural processes such as volatilization, leaf leaching, residue breakdown, and/or root exudate. As a result, it is important to first understand how allelochemicals enter the environment. Allelochemical activity varies according to research methodology and operating processes [10]. The natural state of allelochemicals may be changed somewhat during the process of extraction [11]. As a result, researchers must exercise caution when determining whether a plant has allelopathic potential or separating and identifying allelochemicals using organic solvents and aqueous extracts of plant tissues.

An allelochemical discharged into the environment is typically not a single substance, and the amount released varies depending on the conditions. When investigating plants' allelopathic potential, it is important to evaluate both the type and amount of allelochemicals they release. Interactions such as synergy, antagonism, and incremental effects between different allelochemicals should be investigated because one allelochemical may not exhibit allelopathic

activity as a single component in a given situation but may increase allelopathy when combined with other allelochemicals [12]. Allelopathy is a physiological and ecological mechanism that influences agricultural crop yield [13]. Because of the favourable climatic conditions and socioeconomic relevance of crops in the human diet, crop production is relatively frequent [14]. Plant influences include species, variety, growth stage, and tissue types [15]. Allelopathic effects differ with variety or genotype [16]. Plants from the same environment or with close taxonomic proximity do not always produce secondary metabolites in the same quantities or quality, and thus may not have similar allelopathic effects [17,18]). In a recent study, endogenous allelochemical levels were employed to assess abiotic stress resistance. Meanwhile, exogenous application of allelochemicals has been shown to increase the endogenous level of receivers, resulting in increased growth and resistance to abiotic stresses[19]; thus, appropriate environmental conditions are required for allelopathic studies. According to Albuquerque *et al*. [12], stress can boost the release of allelochemicals from allelopathic plants.

Understanding allelopathy is crucial for understanding its impact on plant germination and seedling growth [20,21]. Germination and seedling growth are critical stages in plant development [22,23] Allelopathy and autoallelopathy are being used to manage organic output in new ways. There is convincing evidence that allelopathic interactions between plants play a crucial role in natural as well as manipulated ecosystems. In recent times evidence is accumulating that all types of plants viz herbs, shrubs and trees, allelopathitically affect the patterning of vegetation, largely in their immediate vicinity. Therefore, in the present work, an attempt has been made to evaluate and compare the allelopathic potentiality of the common agroforestry tree (*C. siamea*) on the germination, seedling growth, and biochemical parameters of two crop species (*A. hypogea* and *V. mungo*).

## MATERIALS AND METHODS

A pot experiment was conducted in the Department of Botany, Annamalai l University, Annamalai Nagar, to assess the allelopathic proclivities of tree leaf extracts on seed germination and growth of ground nut (*Arachis hypogea*) and black gram (*Vigna mungo).* The fresh green leaves of *Cassia siamea* were collected and dried in shed. The leaves were then grinded with the help of a grinder. The grinded material was then soaked in tap water for 5 hrs at room temperature (23 °C). From this stock solution different concentrations ( 10, 20, 20, 40 and 50 g/ L) of extracts were prepared and control crops were used in tap water. The experiment was laid out in a completely randomized design (CRO) and repeated three times.

A sufficient quantity of healthy seeds of two food crops viz. ground nut and black gram were tested for viability and healthy seeds of each food crop were surface sterilized in 15% sodium hypochlorite for 20 min and rinsed several times with distilled water. Ten seeds of each species were sown in pots containing 5 kg of garden soil. The pots were treated with different treatments (concentration) of extract solution and then treated with the respective extracts soon after sowing. The control pots were irrgated with the required amount of water. No fertilizer was applied during the experiment. Data on germination percentage was recorded ten days after sowing and plant height (cm) was recorded 28 days after sowing . Numbers of ground nut and black gram plants that emerged from the soil were counted in each pot and then average was calculated. To record the plant height, crops present in each pot were measured from ground level to the tip of the seedling and then the average was computed.

Experiment was carried out using a Completely Randomized Design (CRD) with 5 treatments, that is, Control (only distilled water); 10 g/L leaf concentration, 20 g/L leaf extract concentration, 30g/L leaf extract concentration, 40g/L leaf extract concentration and 5o g/L leaf extract concentration and each treatment was replicated three times. The number of seeds germinated was counted daily in each treatment and germination was recorded every day till 10 days root and shoot lengths of seedlings were measured and recorded on the 25th day. The experimental data were subjected to analysis of variance (ANOVA). The percentage of inhibition/stimulation effect was calculated using the formula given by Surendra and Pot (1978): $I = 100 - (E2 \times 100/E1)$, where I - % of inhibition/stimulation, E1- the response of control and E2- the response of treatment.

## RESULTS AND DISCUSSION

Allelopathic effects of aqueous leaf extracts of *C. siamea* on seed germination of *A. hypogea* and *V. mungo* (Table-1) showed the stimulatory effects and inhibitory effects on germination. The leaf extract with a higher concentration of (50g/L)*C. siamea*showed an inhibitory effect on seed germination percentage for *A. hypogea* and *V. mungo* but a stimulatory effect was observed at lower concentration extract treatment on both test crops in pot study. At 40g/L extract treatments, the result showed a in the reduction percentage of germination in both test crops (-28.28% and -42%). The variation in germination of different food crops might be due to the variation of species. More days were required for sprouting under higher extract concentration, at the same time positive response was noticed in the 10g/L treatment. The concentration showed greater potential at higher concentrations (Table 1) which can be attributed to the relative amount of allelochemicals released by the extract (El Rokiek *et al*., 2010). The variation in germination of different food crops might be due to the variation in tolerance of the species to different concentrations of the leaf extracts. The inhibitory effect of germination had also been reported earlier by [24] in *Casia occidentalis*. Lower germination may be the result of water uptake inhibition [25], and the disturbance in the synthesis as well as the activity of gibberellic acid (GA3) [26].

Germination was higher in the control and lower concentrations of leaf extracts and water uptake could have played a role in the process. During seed germination, several metabolic and physiological processes could be attributed to water uptake. Reduction in germination percentage has also been reported by some other authors like[27]in wheat treated with *Eucalyptus camaldulensis* leaf extract and [28] in six plant species treated with *Azadiratcha indica* leaf extracts. The allelopathic evaluation in this study showed that the two food species responded to the application of different concentrations of *C.siamea* leaf extracts, as seen by the considerable effects on seed germination. In general, the concentrations of 40g/L and 50g/L caused the maximum inhibition of the germination rate for ground nut and black gram. The effect of 10g/L concentrations of aqueous leaf extracts on plant growth has been explored, and higher concentrations are known to inhibit sprouting [29]. Germination and growth response of the target plants to the allelochemicals may be due to several reasons. Higher concentrations of extract stressed the environment for the test crop which fails in metabolic machinery to activate the embryo to germinate. Lower concentration may stimulate efficient enzyme and hormonal coordination for successful germination. It may be asserted that plants growing in allelochemically induced environment may experience changes in enzyme functionality, water and mineral uptake, permeability of cell membrane and photosynthetic activity corresponding to either reduced germination, seedling growth and dry matter accumulation or stimulation in these parameters [30]. The treatment of 50 g/L was found to be the poorest growth performing treatment of crops. The maximum root length and shoot length reduction were recorded. The positive growth response was observed in both test crops at 10gL-1 treatment of plant leaf extracts. The comparison between treatments and control showed that there was a significant difference. The magnitude of inhibition of root length was considerably greater than shoot length as evident from higher values of response percentage (Table-2). There was significant variation in the root length and shoot length of ground nut and black gram with different concentrations of *C. siamea* leaf extracts.

The plant leaf extracts had an inhibiting effect on root and shoot growth. Statistical analysis revealed that the greatest reduction recorded at the highest concentration had a significant influence on the shoot and root length of groundnut and black gramme overcontrol. However, smaller doses (10 gL-1) of extract treatment in ground nut and black gramme showed significant differences from the control. Allelopathic plants inhibit the growth of nearby plants by influencing their biochemical and physiological processes. Secondary metabolites (allelochemicals) interact with other plants in a variety of chemical interactions, suppressing their growth by influencing plant machinery at the cellular, physiological, and biochemical level. Allelochemical doses and kinds are directly related to their effects on ion absorption. A little quantity of dibutyl phthalate increases nitrogen uptake while decreasing P and K uptake. A considerable amount of this chemical inhibits the absorption of N, P, and K. Similarly, when diphenylamine levels are low, tomato roots absorb N and K more quickly than P [31]. Plants combine phenolic chemicals to modify their defence mechanisms against varied conditions [32].

The inhibitory action of phenol has been attributed to its effect on membrane functions, membrane potential, mineral absorption and plant water relations. This experiment showed that *C. siamea* leaf extracts had more inhibitory effects on the root length and shoot length of *V. mungo* seedlings than in *A. hypogea* crops (Table-2). Stimulatory effects were also noticed at lower concentration ( 10 g/L) treatments in both test crops. As the concentration of extracts increased the inhibition of root length and shoot length also increased. A large number of phytochemicals may be present in the leaf extracts, some of which may be responsible for inhibition and certain for stimulation.

The toxic chemicals of *C. siamea* may inhibit shoot or root growth, they may inhibit nutrient uptake, or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of nutrients [33].A similar trend was observed John *et al*. [34] that fresh leaf extract of mango contains terpenoids and triterpenes, tamarind has flavonoids and terpenoids while teak contains triterpenes. The reduction increased with the increased rates ofresidues. When the crops were treated with *C. siamea* leaf extracts showed chlorohyll reduction in the ground nut and black gram seedlings over control seedlings. The results also showed that chlorophyll content was significantly reduced in 40g/L(chl.a.-29.71%, chl.b. -47.44%, total chlorophyll -39% and carotenoids -20.83% in *A.hypogea* respectively; chl.a. -29.29%, chl.b. -32.10%, total chlorophyll -30.60% and carotenoids -32.01% in *V. mungo* respectively) and50g/L (-42.50%, 55.43%, 49.28% and -39.56% ; -52.22%, -45.21%, -48.94%, -51.09% in *A.hypogea* and *V. mungo* chl.a, chl.b, total chlorophyll and carotenoids respectively) leaf extracts treatments which is accordance with other observations. The reduction in chlorophyll content ultimately resulted in the reduction or decline in photosynthesis in both crop plants.

Chlorophylls a, b, total chlorophyll and carotenoids accumulated in *A. hypogea* and *Vigna mungo* at lower doses ( 10gL-1); the following percentages were observed in both crops; chl.a 4.26% & 4.08%, Chl. b 2.74% & 3.14, total chlorophyll 3.46% & 3.64% and carotenoid contents 0.17% & 0.87% of *C. siamea* leaf extracts treatments over control. However the higher doses (20,30,40 and 50g/L) inhibited seedling chl. a, chl. b, total chlorophyll and carotenoids contents and the effect was concentration dependent. Allelochemicals predominantly influence photosynthesis circuitry in plants, accelerating the breakdown of photosynthetic pigments [35,36]. .Many allelochemicals have been investigated for their effects on photosynthesis in plants. Allelochemicals have been shown to degrade photosynthetic enzymes. These allelochemicals mostly impair Photosystem II (PSII), affecting photosynthesis significantly [37,38]. Sorgoleone is a well-known allelotoxin and lipophilic benzoquinone component that inhibits PSII by limiting the photosynthetic electron transport chain (ETC) [39]. Chlorophylls a, b, and carotenoids accumulated in ground nut and black gramme at lower dosages of C. siamea leaf residues, as did total sugar, primarily the insoluble component. The suppression of photosynthetic pigments at higher concentrations of *C. siamea* leaf extracts was accompanied by a considerable drop in all sugar fractions.

Significant effects of the treatments on Chlorophyll a, chlorophyll b.total chlorophyll and carotenoid contents were recorded with respect to *C. siamea*, on annual crops (ground nut and black gram) as well asthe interaction between tree species and annual crops(Tables 3 & 4). Between the two annual crops, a significant increase was recorded in ground nut while, the decrease was recorded in black gram. Results were found similar to the findings of Venkateshwarlu [40], who has reported inhibition in chlorophyll aand chl. b content (41.66% and 11.36% respectively) in radish when treated with leaf extract of *M. indica*(200 ppm). The reduction in chlorophyll contents observed in the few combinations might be due tothe degradation of chlorophyll pigments or reduction intheir synthesis and the action of flavonoids, terpenoids or other phytochemicals present in leafextracts [41]. Reduction inchlorophylls may decrease photosynthesis and there by substantially decrease all the metabolitesviz., total sugars, proteins and soluble amino acids[42].

Concerning the total phenol content of the annual plants, a significant effect of the treatments could be recorded (Tables 5 & 6). The phenolic content of two seedlings, stimulatory and inhibitory effects was noticed over control. Between the two annual crops, significantly higher values (1.16%) could be found in black gram, when treated with a lower concentration of extract treatment (10g/L) while it was recorded significantly lower in ground nut(-

0.54%).Tables 5 and 6 show that the treatments had a substantial influence on the total phenol content of the annual plants. The phenolic content of two seedlings showed stimulatory and inhibitory effects compared to the control. Compared to the two annual crops, black gramme had significantly higher values (1.16%) when treated with a lower concentration of extract (10g/L), while ground nut had significantly lower values (-0.54%). The largest stimulatory impact was discovered in black gramme (1.16%) when treated with 10g/L. leaf extract, while the maximum inhibitory effect was also obtained in the same crop (-64.33%) when treated with 50g/L extract. This phenolic compound may interfere with the phosphorylation pathway, inhibit the activation of Mg and ATPase activity, or be caused by decreased synthesis of total carbohydrate, protein, and nucleic acid (DNA and RNA), or interference in cell division, mineral uptake, and biosynthetic processes [43].

Significant impacts of the treatment on total soluble sugar and enzyme catalase content of the annual plant were observed for two annual crops, as well as interactions between tree species and annual crops (Tables 5 and 6). The 10g/L concentration treatment resulted in the highest total soluble sugar (0.86% & 0.40% in ground nut and black gramme) and catalase (3.47% & 1.78% in ground nut and black gramme, respectively) content. The observed reduction in total soluble sugar and catalase levels in black gramme is most likely due to photochemical interference in total sugar biosynthetic processes, as validated by Singh and Rao [44] in rice. During the study period, significant effects of treatments on tree species and annual crops were observed.

Allelochemicals affect membrane permeability and integrity [45], plant water relations [46], cell division [47], hormone biosynthesis and transport[48], mineral uptake and transport [49] soil nutrient composition [50], stomatal oscillations [51], and photosynthesis . Phytotoxins can alter plant growth through a variety of mechanisms. They can have an impact on plant biochemistry, physiology, cytology, and morphology, altering its growth and development directly. Regardless of the relationships between plant species, a robust ground for the scientific underpinning of the survival and utility of the allelopathic process should be generalised.

## CONCLUSION

The allelopathic potential of trees and crops can affect tree group growth and division, as well as the yield of vital plants, and allelopathy has proven to be useful in this case. When trees and crops grow together, they form a network that inhibits or stimulates their growth or yield through direct or indirect allelopathic interaction. Allelopathy plays an important function in the ecosystem, affecting plants in both positive and negative ways. The study revealed that the *C. siamea* tree leaf extracts contain allelochemicals, these chemicals affected the studied test crops ie. groundnut and black gram germination, seedling length, chlorophyll, carotenoids, total soluble sugar, total phenol and catalase. This experiment concluded that the allelopathic effect was concentration dependent, and the effect was also different for different species. Between the two crops more allelopathic effect was noticed in *V. mungo* than in *A. hypogea*. Allelopathic connections are a product of secondary metabolism and show diversity in chemical nature and function as a group. There is a need for further study to be carried out on identifying the inhibiting allelochemical in the parts investigated.

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**Table 1. Germination Percentage of groundnut and black gram against *C. siamea***

| Extract concentration(g/L) | *A. hypogea* | *V. mungo* |
|---|---|---|
| Control | 99 | 100 |
| 10 g/L | 100(1.01 | 100(0) |
| 20 g/L | 97(-2.02) | 95(-5) |
| 30 g/L | 86(-13.12) | 79(-21) |
| 40 g/L | 71(-28.28) | 58(-42) |
| 50 g/L | 55(-44.44) | 40(-60) |

Data in parenthesis indicates % increases or decreases

Table-2 **Allelopathic effect of *C. siamea* on root length and shoot length(cm/plant) od *A. hypogea* and *V. mungo***

| Extract concentration | *A. hypogea* | | *V. mungo* | |
|---|---|---|---|---|
| | Root length | Shoot length | Root length | Shoot length |
| Control | 9.166±0.513 | 31.866±0.808 | 7.366±0.450 | 32.4±0.655 |
| 10gL-1 | 9.266±0.514 | 32.466±0.950 | 7.6 ±0.451 | 33.1±0.660 |
| 20gL-1 | 6.266±0.208 | 29.033±0.960 | 6.433±0.503 | 29.8±0.721 |
| 30gL-1 | 5.566±0.251 | 26.866±0.808 | 5.366±0.351 | 25.53±0.503 |
| 40gL-1 | 5.033±0.351 | 24.233±0.208 | 4.866±0.404 | 20.6±0.529 |
| 50gL-1 | 4.66±0.152 | 18±0.866 | 4.1±0.264 | 12.16 ±0.763 |

Means followed by Standard Deviation

**Table-3 Allelopathic effect of *C. siamea* on Chl.a, Chl.b, Total chlorophyll and carotenoids (mg/g. fr.wt.) contents of *A. hypogea***

| Extract concentration | Chlorophyll a | Chlorophyll b | Total chlorophyll | Carotenoids |
|---|---|---|---|---|
| Control | 0.727 | 0.801 | 1.528 | 0.599 |
| 10gL-1 | 0.758(4.26) | 0.823 (2.74) | 1.581(3.46) | 0.676(0.17 |
| 20gL-1 | 0.632(-13.06) | 0.687 (-14.23) | 1.319(-13.67) | 0.66(-3.17) |
| 30gL-1 | 0.561(-22.83) | 0.54(-32.58) | 1.101(-27.94) | 0.564(-5.84) |
| 40gL-1 | 0.511(-29.71) | 0.421(-47.44) | 0.932(-39.00) | 0.476(-20.83) |
| 50gL-1 | 0.418(-42.50) | 0.357(-55.43) | 0.775(-49.28) | 0.362(-39.56) |

Data in parenthesis indicates % increases or decreases

**Table - 4 Allelopathic effect of *C. siamea* on Chl.a, Chl.b, Total chlorophyll and carotenoids (mg/g. fr.wt.) contents of *V. mungo* on 28th Day old seeding**

| Extract concentration | Chlorophyll a | Chlorophyll b | Total chlorophyll | Carotenoids |
|---|---|---|---|---|
| Control | 0.833 | 0.732 | 1.565 | 0.456 |
| 10gL-1 | 0.867 (4.08) | 0.755(3.14) | 1.622(3.64) | 0.479(0.87) |
| 20gL-1 | 0.761(-8.64) | 0.698(-4.64) | 1.459(-6.77) | 0.438(-3.94) |
| 30gL-1 | 0.69(-17.64) | 0.591(-19.26) | 1.281(-18.47) | 0.389(-14.69) |
| 40gL-1 | 0.589(-29.29) | 0.497(-32.10) | 1.086(-30.60) | 0.31(-32.01) |
| 50gL-1 | 0.398(-52.22) | 0.401(-45.21) | 0.799(-48.94) | 0.223(-51.09) |

Data in parenthesis indicates % increases or decreases over control

**Table-5 Allelopathic effect of *C. siamea* on Total soluble sugar, Phenol and catalase (mg/g. fr.wt.) contents of *A. hypogea* on 28th Day old seeding;**

| **Extract concentration** | **Total soluble sugar** | **Phenol** | **catalase** |
|---|---|---|---|
| Control | 1.267 | 0.923 | 1.094 |
| 10gL-1 | 1.278(0.86) | 0.918(-0.54) | 1.017(3.47) |
| 20gL-1 | 1.202(-5.13) | 0.863(-6.50) | 0.901(-17.64) |
| 30gL-1 | 1.123(-11.36) | 0.789(-14.51) | 0.845(-22.76) |
| 40gL-1 | 0.87(-31.33) | 0.603(-34.66) | 0.699(-36.10) |
| 50gL-1 | 0.698(-44.90) | 0.388(-57.96) | 0.436(-60.14) |

Data in parenthesis indicates % increases or decreases over control

**Table - 6 Allelopathic effect of *C. siamea* on Total soluble sugar, Phenol and catalase (mg/g. fr.wt.) contents of *V. mungo* on 28th Day old seeding.**

| **Extract concentration** | **Total soluble sugar** | **Phenol** | **catalase** |
|---|---|---|---|
| Control | 0.988 | 1.901 | 0.895 |
| 10gL-1 | 0.992(0.40) | 1.923(1.16) | 0.871(1.78) |
| 20gL-1 | 0.91(-7.89) | 1.796(-5.52) | 0.755(-15.64) |
| 30gL-1 | 0.79(-20.04) | 1.509(-20.62) | 0.623(-30.39) |
| 40gL-1 | 0.572(-42.10) | 0.992(-47.81) | 0.474(-47.03) |
| 50gL-1 | 0.334(-66.19) | 0.678(-64.33) | 0.234(-73.85) |

Data in parenthesis indicates % increases or decreases over control

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**RESEARCH ARTICLE**

# Management of Ocular Insect bite through Ayurveda- a Case Study

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## ABSTRACT

The origins of *Visha* and *KeetaVisha* were recited by *Acharya Charaka* in *Chikitsa sthana* of *Charaka Samhita, Acharya Sushruta* in *Kalpasthana* of *Sushruta Samhita*, and *AcharyaVagbhata* in *Uttartantra* of *Ashtanga Hridaya*, respectively. *Acharya Sushruta* explained *KeetaVisha* as *Jangama Visha* (Animate Poison) and classified 67 different types of *Keeta* into four groups that may cause both acute and severe manifestations in humans. The most common symptoms of an insect bite are itching, a burning sensation, swelling, and pain at the bite site. The diagnosis and treatment of *Keeta visha* differs according to thetypes of *Keeta*, so they are frequently misdiagnosed because the signs and symptoms resemble those of a skin disease.This is a case report of a 32-year-old female who had a history of an unknown insect bite and presented to the eye OPD with complaints of swelling on both upper eyelids, itching in the periorbital area, burning sensation in both eyes, pain opening her eyes and watering from both eyes. *Acharya Sushruta's Kalpasthana* contains numerous formulations for all types of *Visha*. As a result, the patient was treated with *Aschyotana (Aatyayik chikitsa), Bilvadi Gutika*, *Punarnava Guggulu* and *Manjishthadi Kashaya*, as well as *Bidalaka*, a local application with *Vishaghna* properties. In this case, drugs have provided reassuring results in the treatment of an unknown insect bite.

**Keywords:** Aschyotana, Ayurveda,insect bite, Keeta, Nayanabhighata, Ocular emergencies

# INTRODUCTION

*Netrabhighata* (Ocular injury)can occur by either director indirect causes. As *Acharya Dalhana* mentioned, direct causes include- direct shock or blow, foreign body, *vayu*, dust, smoke, touch of insects, *Tikshnanjana*, over fomentation of eyes, injury due to water force while swimming, rubbing of the eyes vigorously, staring at celestial bodies like sun with naked eyes [1]. According to *Charak Acharya*, insects are also known as *Keeta* because they are produced from *Kitta* or waste products such as stool and urine [2]. The term *Keeta* refers to all living creatures which are small in size but visible, having two or more legs with or without wings and stings are present, which is included in *Jangamvisha* [3]. An insect bite to the eyelid usually results in redness and inflammation of the eyelid and surrounding area. Fluid accumulation and inflammation are common after an insect bite due to the loose tissue surrounding the eye [4]. In severe cases, it can even prevent the eye from opening, especially when lying down, because the fluid gravitates to that area. Because the skin around the eye is sensitive, the itching and discomfort caused by a bite on the eyelid may feel especially intense; however, most of the time, the itchiness lasts only a few days. Symptoms may include swelling, redness of the eyes, pricking or burning pain, and tenderness to touch occurring around one or bilateral eyes. The affected person can move the eye in any direction without pain, but opening the eyelid can be difficult, often due to swelling. The significant variation in clinical presentation and outcomes between several cases has made it difficult to develop a therapeutic algorithm.

**Aim**

To evaluate the efficacy of *Ayurveda* therapy in the management of ocular insect bite.

**Objectives**

- To study on insect bite in detail.
- To study the probable mode of action of *Ayurveda* management of ocular insect bite.

# MATERIALS AND METHODS

**Case Report**

A 32 years old female, resident of Vadodara reported to *ShalakyaTantra* Eye OPD of Parul Ayurved Hospital, Vadodara with following complaints since 1 day:

- Swelling on bilateral upper eyelids and forehead
- Burning sensation in bilateral eyes
- Itching at periorbital area
- Pain while opening the eyes
- Watering from bilateral eyes

Patient was vitally stable and her appetite was moderate.

**Past History**

History of unknown insect bite on both upper eyelid 1 day back.

**Ocular examination**

Swelling over the forehead (glabella).No evidence of any inflammatory changes. After keen ocular examination with slit lamp, no extra ocular foreign body was found.

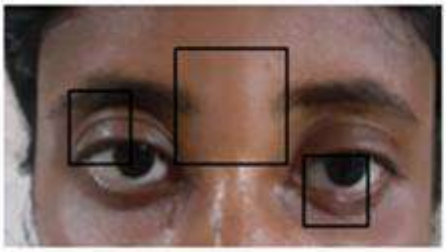
**Systemic Examination**

BP- 110/80 mmHg
Pulse- 83/min
T- Afebrile
RR- 18/min

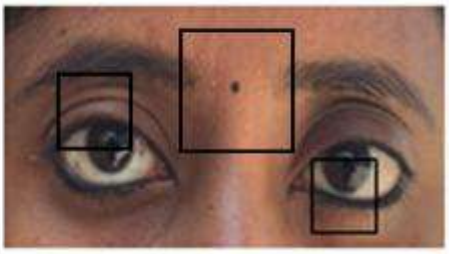
**Treatment Plan**

The main aim of treatment is to give relief to symptoms, avoid further complication and positive psychological approach to the patient. *Aschyotana* with *Yashtimadhu Ghrita* was done for 7 days as *Aatyayik chikitasa*(Emergency treatment) [5]

**OBSERVATION & RESULTS**



**Before Management**



**after Management**

Gradation: Table 3: Gradation of the symptoms
Results: Table. 4

## DISCUSSION

Despite the fact that the lids, eyelashes, and orbital margins protect the eye, *Neeja* or *Agantuj* cause can injured the eyes in a variety of ways. The most feared outcome of the *Netrabhighata* is vision loss, which must be treated immediately.During *Abhighata*, the *Doshas Rakta* and *Pitta* are the most affected. *Abhighata* causes *Pitta* and *Rakta* to become vitiated, followed by *Vata* and *Kapha* to become vitiated, resulting in the formation of *Sopha, Puya, Bhedha* of *Twacha* and *Vrana* [6]. Therefore, *Tridoshahara Pathyas, Snighdha, Hima* and *MadhuraDravyas* are recommended to combat the negative effects of vitiated *Rakta* and *Tridosha* while also soothing the eyes.

The probable mode of action of the medicines used in this case is as follows:
***Yashtimadhu Ghrita:*** It includes *Yashtimadhu* and *Ghrita*. Both ***Yashtimadhu*** and ***Ghrita*** contain *Madhura Rasa, Guru-Snigdha Guna, Madhura Vipaka* and *Sheeta Virya*. It calms the agitated *Vata* due to its *Madhura Rasa, Guru-Snigdha Guna* and *Madhura Vipaka*. It also soothes the irritated *pitta* with its *Madhura rasa, Madhura Vipaka* and *Sheeta Virya*. It has healing, skin regeneration, and anti-inflammatory properties. It also owns *Rasayana* and *Ropana* properties. ***Yashtimadhu***: The antioxidant activity of G. glabra is one of the primary reasons for its use.The phenolic content is most likely responsible for the high antioxidant activity observed. The responsible compounds are primarily isoflavones, including glabridin, hispaglabridin A, and 30-hydroxy-4-O-methylglabridin. The dihydrostilbene derivatives found in G. glabra leaves have high antioxidant activity. G. glabra also contains licochalcones B and D, which have strong DPPH radical scavenging activity and can inhibit microsomal lipid peroxidation. These phenolic compounds are effective at protecting biological systems from oxidative stress and inhibiting the onset of skin damage. The topical application of liquorice extract formulations may be beneficial in innovative dermal and cosmetic products because it counteracts oxidative stress damage and maintains skin homeostasis due to its high antioxidant content. The antibacterial activity observed is due to the presence of secondary metabolites, namely, saponins, alkaloids, and flavonoids [7].

***Bidalaka***: According to *Srotomaya Purusha*, the entire body is made up of *SukshmaSrotasa*, or it is porous. Through these pores or channels, minute particles of drug applied in the form of *Bidalaka* penetrate the skin. At this stage, the *UpshoshanaGuna* of *Vata Dosha* aids in drug penetration and absorption. *Bhrajaka Pitta*, which is present in the skin, is responsible for the metabolism of drugs applied to the skin [8,9]. The drugs used in *Bidalaka* promote wound healing.

It includes *Yashtimadhu, Amalaki, Haritaki, Daruharidra, Lodhra* which contains *Vrana Shodhana-Ropana* (woundhealing)*Guna, Shophaghna* (anti-inflammatory), *Indriyabalaprada* (immunomodulatory), *Raktaprasadana* (blood purification), and *Dahaprashamana* (reduce burning sensation), *Tridoshaghna* as well as *Rujahara* (analgesic), and *Kandughna* (anti-pruritus)*Guna*. *AcharyaCharaka* classifies *Lodhra* as *Sandhaniyagana Dravya* (wound-healing drugs). It includes *Kashaya-Tikta rasa, Laghu-Ruksha guna, Katu Vipaka* and *Sheeta Virya*. Thus, it balances *kapha* and *Pitta*. Ethanolic extract present in *Lodhra* is responsible for analgesics and anti-inflammatory activities [10].

***BilvadiGutika Vati***: ***Bilva-***It includes *Laghu Guna, Sheeta Virya* and *Madhura Vipaka*. It contains *Mutrala* (diuretic), *Tridoshaghna*, *Shothahara* (anti-inflammatory), *Vedanasthapana* (analgesic), *Raktastambhan* (hemostatic), *Deepana* (appetiser), *Pachana* (digestive), and *Grahi* (astringent) properties. According to studies, the pulp of the *Bilva* fruit contains a variety of bioactive substances, including carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids, and terpenoids which are responsible for anti-inflammatory, anti-genotoxic, anti-bacterial, anti-fungal, and antioxidant properties. Chemical structures of the compounds present in *Bilva* areCitral (antibacterial, antifungal, and antiparasitic), Cumin aldehyde (Insecticide), Eugenol (antibacterial, analgesic, and antioxidant), rutin ( Antioxidant, Anti-inflammatory), β sitosterol (Antioxidant). [11]. It has been found to have healing properties and is beneficial for blood purification.[12]Because it balances *Kapha* and *Vata*, it is effective in treating pain and swelling, as well as managing various inflammatory changes in the body. ***Surasa*** has anti-inflammatory, analgesic, and antipyretic properties, as well as immunoregulatory action. It also acts as an antihistamine, antibacterial, and has antitoxic properties.

***Karanja, Haridra, Daruharidra-*** These medications include *Tikta-Katu Rasa, Ruksha-Laghu Guna, Katu Vipaka* and *Ushna Virya*. It balances *Vata* and *Kapha* due to *Ushna Virya*; *Ruksha-Laghu Guna* pacifies *Kapha*; and *Tikta Rasa* pacifies *Pitta*. Hence, it balances all three *Doshas*. It reduces skin lesions and effectively relieves the itching sensation associated with them. ***Tagara*** has *Katu-Tikta-Kashaya Rasa*, *Laghu-Snigdha Guna*, *Katu Vipaka* and *Ushna Virya*. It possesses *Kapha Vata Shamaka* and *Vishaghna* properties. *Haritaki* includes *Lavana Varjita Pancharasa*, *Laghu-Ruksha Guna*, *Madhura Vipaka* and *Ushna Virya*. It's called *Vatanulomaka* and it's primarily responsible for interrupting the progression of inflammation and pain. ***Pippali*** contains *Katu Rasa*, *Laghu-Tikshna Guna*, *Madhura Vipaka* and *Anushna-Sheeta Virya* all of which may act as a *Pitta*-reducingdrug***. Sunthi*** has *Katu Rasa, Guru-Ruksha-Tikshna Guna, Madhura Vipaka* and *Ushna Virya*, which calms *Kapha*. It is used to treat bothacute andchronic inflammation, skin infections, and oxidative stress [13]. *BilvadiGutika* performs *Aam Pachana*, which helps to eliminate *Gara Visha* and *Agni Mandhya*.

***Punarnavadi Guggulu***: ***Punarnava*** contains *Madhura-Tikta-Kashaya Rasa, Laghu-RukshaGuna, Katu Vipaka* and *Ushna Virya*. It balances the *Kapha* and *VataDosha* and performs *Sothaghna* and *Rasayana* actions. It contains several bioactive chemical constituents, including punarnavine, isoflavonoids (rotenoids), sitosterol, an alkaloid (boeravinone), eupalitin, beta-sitosterol, and palmitic acid. [14]. These active chemical constituents are found in various parts of the plant and have pharmacological and therapeutic properties such as immunomodulation, anti-inflammatory, antioxidant, antifungal, anti-microbial, anti-histamine, and anti-stress [15].

***Devdaru*** includes *Tikta-Katu-Kashaya Rasa, Ruksha-Laghu Guna, Katu Vipaka* and *UshnaVirya*. It contains *Vatahara* and *Sophaghna* properties. ***Haritaki*** includes *Shothahara, Deepana*, *Pachana* and *Shulahara* properties. ***Guduchi*** contains *Kashaya-Tikta Rasa*, *Laghu-Snigdha Guna, Madhura Vipaka* and *Ushna Virya*. *Guduchi* is *Tridoshaghna* and performs *Rasayana* and *Dhatvagni Vardhaka* actions. ***Guggulu*** includes *Tikta-Katu Rasa, Laghu*, *Ruksha, Vishada Guna, Sookshma, Sara Guna, Katu Vipaka*and Ushna Virya. It helps to resolve *Shotha Samprapti***.**

***Manjishthadi Kashaya***: ***Manjishtha*** consists of *Guru-Ruksha Guna*, *Tikta-Kashaya-MadhuraRasa, Katu Vipaka* and *Ushna Virya*. It possesses *Kapha-Pitta-Shamaka* properties. Phenolic compounds were identified in *Manjishtha* through phytochemical testing. Phenol is an effective antiseptic for preventing infectious pathologies. Tannic acid solution acts as a chemical antidote, precipitating poisons such as alkaloids and metals. *Manjishtha* also contains carbohydrate, alkaloids, amino acids, saponin, glycosides, and tannins, making it a medicinal phytochemical with antitoxic, detoxifying, antiseptic, antimutagenic, anticarcinogenic, and antioxidant properties [16]. It has anti-inflammatory,

pain-relieving, and antimicrobial properties. ***Triphala*** has antimicrobial and anti-inflammatory properties. ***Vacha*** has anti-inflammatory, immunomodulatory, antibacterial, and anti-ulcer properties. ***Nimb*** has *Tikta-KashayaRasa, Laghu-Ruksha Guna, Katu Vipaka*, and *Sheeta Viryas*. It has antibacterial, anti-inflammatory, antifungal, antioxidative, and immunomodulatory properties. All of these medications have anti-inflammatory, anti-oxidant, wound healing, antibacterial, antifungal, and antimicrobial properties that help with early wound healing and prevent complications.

## CONCLUSION

Every ocular structure is prone to injury. The site is frequently determined by the cause and mechanism. It can range from minor injuries, such as getting dust in your eyes, to major injuries that cause permanent vision loss. Because of its negative consequences, our *Acharyas* have given detailed explanations for *Nayanabhighata*. In this case, the *Chikitsa* is determined by the *Avastha* and *Dosha* predominance. Our *Acharyas* have explained various treatment modalities such as *Nasya, Alepa, Parisechana, Tarpana*, the concept of *Pathya*, *Drushtiprasada JananaDravyas*, and the use of *Snigdha, Hima, and Madhura Dravyas* for various types of *KeetaVisha*.[17]The signs & symptoms of the patient were improved after four days of treatment and was completely relieved within seven days. Based on the findings of this clinical case study, we can conclude that *VishaghnaAushadhipana* combined with *Vishaghna Lepa (Bidalaka)* can provide a reliable and quick result with no local or systemic side effects. Ocular therapeutic procedure in the form of "*Aschyotana"*also played major role in the primary and emergency management*(Aatyayik chikitasa)*towards the symptoms of ocular injury.

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**Table 1: Ocular examination**

| | **Right eye** | **Left eye** |
|---|---|---|
| **Eyelashes** | NAD | NAD |
| **Eyelids** | Painful swelling ++ | Painful swelling +++ |
| **Conjunctiva (Bulbar)** | NAD | NAD |
| **Conjunctiva (Palpebral)** | Mild congestion + | Mild congestion + |
| **Cornea** | Clear | Clear |
| **Sclera** | NAD | NAD |
| **Pupil** | Round, Regular, Reactive to light | Round, Regular, Reactive to light |
| **Lens** | Transparent | Transparent |
| **Visual Acuity** | 6/6 | 6/6 (p) with pinhole 6/6 |

**Table 2: Treatment protocol**

| Name of procedure /Drug | Dosage | Route of administration | Duration |
|---|---|---|---|
| *Aschyotana* with *Yashtimadhu Ghrita* | 10-10 drops in bilateral eyes | Topical | 7 days |
| *Bidalaka (Yashtimadhu, Amalaki, Haritaki, Daruharidra, Lodhra )* | 5 gm each | Local application | 7 days |
| *Bilvadi Gutika* | 2 tablets twice a day with luke warm water after meal | Oral | 7 days |
| *Punarnavadi GugguluVati* | 2 tablets thrice a day with luke warm water after meal | Oral | 7 days |
| *Manjishthadi Kashaya* | 15 ml Kashaya with 45 ml water Twice a day after meal | Oral | 7 days |

**Table 3: Gradation of the symptoms**

| Symptoms | Grade |
|---|---|
| Severe | +++ |
| Moderate | ++ |
| Mild | + |
| No symptoms | 0 |

**Table 4: Result**

| Sign & symptoms | Before treatment | Day 4 | Day 7 |
|---|---|---|---|
| Swelling over glabella | +++ | ++ | 0 |
| Oedematous eyelids | ++ | + | 0 |
| Itchingat periorbital area | ++ | 0 | 0 |
| Congestion on palpebral conjunctiva | + | 0 | 0 |
| Burning sensation in eyes | +++ | + | 0 |
| Tenderness on touch | +++ | ++ | 0 |
| Watering from bilateral eyes | + | 0 | 0 |

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**RESEARCH ARTICLE**

# Polyherbal Face Pack Development, Formulation Techniques and Evaluation Study

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**ABSTRACT**

The aim of this research work is to formulate and evaluate polyherbal face pack for cosmetic purposes from herbal ingredients. Multani mitti (Fullers earth), sandalwood, orange peel, and almond peel are the medicinal plant materials used as traditionally from ancient years in herbal medicines such as Ayurveda, Homeopathy and Siddha. These ingredients either purchased from local market or from flipcart, sieved using #40 mesh under sieve number 120. Mixed well and kept in air tight container for further evaluation. Bulk density, tapped density, moisture content, total ash angle of repose, particle size, irritancy test were performed. Shinnoda test and organoleptic evaluation were also carried out. Out of three formulations F1 was found to be very effective.

**Keywords:** Face pack, Multani mitti, orange peel, sandalwood, evaluation.

## INTRODUCTION

Each one wants to get fair and attractive skin. Now a day, pimples, dark circle Acne, black head are common among youths who suffers from it. As per the Ayurvedic system of medicine, problems of Skin are generally because of

impurities in blood. Accumulated pollutants in the blood during unhygienic food and lifestyle on daily basis can cause skin related diseases. Several herbs, remedies are designed in Ayurvedic system for blood cleansing. Herbs alike Chandana, Haldi, Manjistha, Lodhra, etc. are convenient example for blood purifier. The herbal pastes which are useful on facial skin to cure acne, pimple, scars, marks or pigments are known as "mukha lepa" in Ayurvedic system. The method of applying this herbal mixture on facial skin is termed as "mukha lepana". This particular beautifying therapy is common as facial. The smoothing powder which is used in appliaction on facial skin is termed as "face pack". A decent herbal face pack must into supply of essential nutrients onto skin. It should enter the subcutaneous tissues in ordering of to deliver the essential nutrients. Dissimilar types of skin requires several types of herbal face pack [1]. A face pack is a powder that smoothes and nourishes the skin on the face. A good herbal face pack should also contain the nutrients that the skin needs to penetrate the subcutaneous tissues and reach the skin's surface. Ayurvedic face packs are supposed to help minimize wrinkles, acne, pimples, and dark bags under the eyes. Additionally, a face pack brightens and smoothes the skin [2].

Cosmetics are products used in skin and hair care regimens, primarily by young people, with the aim of improving, beautifying, and cleaning the qualities that make them beautiful. Skincare treatments are not popular right now. In actuality, cosmetics are used by people in every civilization to improve or protect their skin, which undoubtedly proves that this is a growing demand. The conventional idea of applying cosmetics to highlight the characteristics of improved health has not altered, despite the fact that cosmetics have undergone several alterations in more recent times [3]. The smoothing powder that is currently applied to facial skin is called a face pack. Using powders or pastes, these preparations of different combinations are applied to the face skin and allowed to dry and set to form a film that tightens, strengthens, and cleanses the skin. In order to get the resulting layer of face pack, which contracts and hardens and is easily removed, face packs are typically kept on the skin for fifteen to thirty minutes to allow all of the water to evaporate. Although the colloidal and adsorption clay combinations used in these processes remove excess oil and filth from the facial skin, the softening and tightening impact that follows the administration of a face pack gives the impression of a renewed face. When the face pack is eventually removed, the injured skin and accumulated debris are also eliminated [4].

A homemade face pack using only natural products and masks renders skin more luminous, smooth, and soft [5]. The development, description, and application of a natural herbal face pack that is pure cosmetic is the focus of this research article today. Normal skin seems lively and moisturized since it is neither exceptionally oily nor dehydrated. Cleansing, toning, and moisturizing are the steps in a routine skin care treatment. However, the herbs included in this recipe revitalize the skin to prevent it from becoming allergic and sunburned. Present research work was carried to frame herbal face pack comprising powders of Fullers earth(also known as multani mitti) , green tea, turmeric, almond, orange peel, sandalwood as the required ingredients which will accomplish as complete face pack .This prepared face pack was evaluated furthur by physical, chemical and biological evaluation [6].

**Benefits of Applying Face Pack** [7]

1. Helps in the reduction of acne, pimple, scars and marks depending on the herbal ingredients we use.
2. These face packs produces a gentle and relaxing effect on the facial skin.
3. Face packs mainly removes the dead cells of facial skin.
4. Fruit face packs provides vital nutrient to skin
5. On application of natural face masks on daily basis brings glow to skin, improve skin quality and skin tone.
6. These face packs advantages us to reappearance the lost glow and shine of skin in short period of time.
7. Face pack helps to avoid early aging of skin.
8. The use of face packs judicial can effectively opposed the injurious effects of pollution and severe climates on skin.
9. Natural face packs make the skin look beautiful and full of essential nutrition.
10. Development of wrinkles, fine lines and sagging of skin can be well prevented by using of natural face packs.

**Precautions to be Taken While Applying Face** [8].

1. The face pack should be removed within 15 to 20 minutes from face. Keeping face pack for extended time period may result in development of wrinkles, sagging of skin and increase of open pores.
2. Choose the face pack giving preference to your skin type. Take views of natural therapist or concerned skin professional before application of face pack.
3. Before removing of dried face pack, spray water(which should be at room temperature). After getting rid of the mask, rub an ice cube on facial skin. This would help us to close open pores and constricts the skin. will also tones and sooths the skin
4. Use of face pack once in a week. Never try to scratch or peel the dry face pack. This may harm original skin.
5. Prevent using face pack nearby of "eye zone". The skin nearby eye is very gentle. The process of washing face pack may harm skin around eyes.
6. Never rub face forcefully. This may result in outbreak of pimples and dark spots. When applied face pack stay away from heat.

## MATERILAS AND METHODS

**Plant Materials:** The herbal materials utilized in this study were either bought from a flip cart (green tea, turmeric), or they were obtained at the local market (Multani mitti, Sandalwood, Orange peel, and Almond peel powder). The herbs were then dried and pulverized for later usage. The information about the plant materials used in the formulation of the face pack is provided below.

**Multani Mitti (Calcium bentonite**)

Calcium bentonite benefits skin in a variety of unique ways, including reducing the size of pores, getting rid of whiteheads and blackheads, fading freckles, calming sunburns, cleaning the skin, improving skin tone, improving blood circulation, treating acne, and giving skin a radiant appearance due to its high nutrient content. Multani mitti is a great remedy for bad and irritated skin and will repair your skin to make it glow. Magnesium chloride is abundant in Multani Mitti [9]. The word "fullers earth" comes from the initial procedure of using a fullers earth slurry mixed with water to clean or pull wool in order to get rid of dirt and oil. Because it improves blood circulation, relaxes and smoothes the skin, it is also a component of cosmetic goods. Because it is a pure and natural cleansing agent, it improves the skin's shine [10].

**Turmeric (*Curcuma longa*)**

Curcuma longa has anti- allergic and anti-inflammatory activity. It helps in wound healing and best blood cleanser. It provides best blood purification action hence it is used in almost disease having properties with blood impurities origin. Haridra is renew of skin and helps to make skin again youngby delaying the symbols of aging likewise wrinkles [11].Turmeric Powder, termed from *Curcuma longa*, is primarily used as an antibacterial, antiseptic, and anti-inflammatory properties. Curcuma longa also acts as a blood cleanser and well treats the acne by fighting against pimples and decreasing oil secretion from the sebaceous glands [12].

**Sandalwood (*Santalum album*)**

*Santalum alba* has an anti-aging and anti-tanning property. Sandalwood keeps the skin cool, fair and healthy by defending the skin from the effect of environmental pollutants. Sandalwood is helpful Ayurvedic herb with antimicrobial properties and is used for curing several skin problems and eliminates scars [13]. It is a herbal species obtained from plant in the family of Santalaceae of genus Santalum hence both commercially and ethnically valuable. The oil contained in the sandalwood wooden, primarily in the heartwood, is the important reason for its cultural and commercial rank. If your skin is greasy, you can use it to get rid of dark spots. Anti-ageing and anti-tanning values found in *Satalum album*. It is also beneficial for the skin in a several of ways composing of toning, soothing, antibacterial, calming and healing characteristics [14].

**Orange peel (*citrus aurantium)***

Orange peel powder, carried from Citrus reticulate, is a citrus fruit valued in nutrients like magnesium, calcium, potassium, and vitamin C. It shields the skin from oxidative stress, provides hydration, and reduces free radicals. Moreover, it imparts an instantaneous radiance to the skin, prevents wrinkles, blemishes, acnes, and signs of aging

[15]. On top of fighting free radicals, vitamin C brightens your skin and makes it younger and shining. Orange peel powder helps in including unclogging pores, removing blackheads and treating acne. It comprises citric acid, which remove dead skin cells [16].

**Green tea powder**

It is belongs to family theaceae and used as anti-aging, anti-inflammatory and healthy glow for skin. Green tea is now a focus of interest as of its confirmed anti-oxidant properties or its capability to reparation of UV photo damage and toxicity due to light. This action of green tea is supposed to be because of catechins that are the most vital polyphenols in green tea [17]. Newly, the lotus *Nelumbo nucifera's* leaf, seed, and flower extract were examined for anti-wrinkle properties and presented 56%, 49%, and 54% inhibition of elastastase [18].

**Almond peel powder**

Family: Rosaceae Use: Cleanser, scrub and moisturizer in an earlier examination, the *Prunus amygdalus* L. extract (skin) was used in herbal formulation and further studied for skin protection from premature aging of skin caused by UV radiations of sun. Particular antioxidant activity was revealed by the mice skin afterward treatment, as malondi aldehyde (MDA) level was decreased, whereas level of Glutathione (GSH) was increased after treatment [19].

**Methodology**

**1. Bulk density (B.D)** [20]

It is the proportion of total mass of powder upon the bulk volume of powder .It was quantify by sheeting down the weighed powder (passing through standard sieve#20) inside the measuring cylinder further the initial volume called the bulk volume, from here one can calculate bulk density according to formula given below: should be expressed in g/cc and given by.

$B.D = M/V_0$

Where, M=mass of the powder

$V_0$= bulk volume of the powder.

**2. Tapped density** [21].

Tapped density is an increased bulk density which is accomplished just after mechanically tapping of the vessel containing the powdered sample. Next witnessing the initial powder volume or mass, the measuring cylinder or container tapped mechanically for 1 min further volume and mass readings are noted until little more mass change or volume change was noted down, hence expressed in grams per cubic centimetre.

Tapped density of formulation should not be more than 0.5 g/cc. Tapped density = Mass/Tapped volume.

**3. Moisture content** [22].

Take a thin ceramic dish and weigh around 1.5 g of the powder drug in it. Keep the dish in oven at 100 degree Celsius to get it dry, while waiting for two repeated weighing do not vary by additional than 0.5 mg. Cool and weigh the powder dish, further the loss in weight is typically noted as moisture content. Moisture content not more than 4% w/w.

LOD (Loss on Drying) can be calculated by using following formula.

% LOD = Initial sample wt. – wt. of dried material / total wt. of drug taken x 100.

**4. Angle of repose**

The method uses to calculate angle of repose was fixed funnel method. Powder was transferred over a funnel to form a cone shape structure. As the growing cone, the tip of the funnel must be held nearby and slowly raised as the pile grows so that to decrease the effect of falling particles. Further estimate the height and radius of the pile.

$\theta = \tan^{-1} (h / r)$

Where, $\theta$ = Angle of repose

h = Height of the heap

r = Radius of the base

**5. Ash Value**

Ash value is also termed as total ash. In the evaluation of pharmaceuticals and herbal drugs ash value is a key parameter. The particular known weight of sample was permitted to burn or destroy totally in a standard muffle furnace. The residue which is left in the form of ash comprises of salts, metals and other non-combustible materials termed as inorganic mineral components which was evaluated further.

**a)Total Ash Value**

The situation is not possible to known the drug identity or purity only from the ash content, but then again it can provide the visions into the quality of the sample. Accurately weigh 2g of sample in the crucible and further note the weight of it. Place the crucible for 24 hours in the standard muffle furnace set at 450 degree of Celsius than turned off the muffle furnace and allowed to cool it. Further remove the crucible from muffle furnace and weighed the as sample and note down as crucible plus ash sample (total weight).

Weight of Ash (mg) = (weight of crucible + Ash) − (weight of crucible)

Ash (%) = Weight of ash (mg)/ Initial sample weight × 100

**b)Acid Insoluble Ash Value**

Add 25 ml of HCL to the crucible containing total ash, and cover it with a watch glass. Than boil the mixture for 5 minutes. Further wash the watch glass with hot water (5ml) and added into the crucible. The ash-less filter paper was rinsed with hot water till it become neutral in which insoluble matter was collected. The filter paper comprising the insoluble matter was removed to the original crucible and desiccated on a hot plate. The sample with the crucible was burnt to a constant weight and left in the desiccator to get cool for 30 minutes. After cooling, the sample was weighed. The value or percentage of acid-insoluble ash was noted down in reference to the air-dried sample.

Acid Insoluble Ash Value = Weight of acid insoluble Ash/ Weight of air dried sample x 100

**8. PH**

An electrode pH meter used single standard or double electrode. By using proper buffer solution, instrument must be firstly calibrated at pH 7 and 9.2. The test sample consisting of 10 percent (m/v) dispersion of the product of either type of in previously boiled and cooled water shall be poured into a glass beaker and pH determined directly without any dilution within 5-10 minutes. Digital pH meter was used to measure the pH.

**9. Particle size**

Particle size is a general property of a powder. That affect several properties likely grittiness, spread ability etc. particle size was known using sieving method byI.P. Standard sieves through mechanical shaking for 10 min.

**10. Shinoda test**

Firstly prepare the ethanolic extract of using your sample powder than add few drops of concentrated hydrochloric acid (HCL) to it. At that point add magnesium turnings into the solution and appearance of pink, red colour tells that flavonoids are present in the sample powder.

**Formulation of Herbal Face Pack**

The herbal face pack was framed using basic mixing process. Herbal face pack was prepared or formulated by additionof all the essential amounts of herbal ingredients as given in formulation table1.

**Method of preparation** [23].

The crushed constituents used for herbal face pack were sieved using #40 mesh under sieve number 120

↓

Ingredients were weighed precisely and mixed geometrically for even mixing.

↓

This was then kept in an air close-fitting container for evaluation

**Application procedure for face pack**

Take required face pack powder in a container as per the necessity and add rose water to mix. Mix well and spread over the facial skin. Cover properly the acne and marks, spots too. As kept it for whole drying for 10 to 15 min and then cleanse with cold water

**Evaluation parameters** [24].

**Organoleptic Properties:** The dried powder nature, color, odour, taste and texture were tested manually in combined form.
**Physicochemical Evaluation:** pH was determined by performing under PH meter, total ash and acid insoluble ash was completed by using incinerator and moisture content was also executed.
**General powder Characteristics:** By using standard sieving method the particle size of combined dried powder was perform. Angle of repose by method of funnel, bulk density and tape density was evaluated by the flow property of the dried powder of combined form.
**Shinoda Test:** To the extract prepared of ethanol add little magnesium turnings and drops of concentrated HCL. The arrival of red or pink colour specifies the presence of flavonoids

## RESULTS AND DISCUSSION

**Organoleptic evaluation** Face pack was formed and estimated for organoleptic parameters shown in the Table 2. The free flowing properties were shown by flow property parameter. The formation of colour was minor yellow. The fragrance of prepared formulations was good satisfactory which is required as cosmetic preparations. The smoothness and Texture and Smoothness was also good acceptable which is desirable as cosmetic formulations
**Physical evaluation**
The particle size was tested by standard sieving method. The angle of repose using funnel method, bulk density and tapped density by tapping method was evaluated by flow property of the dried powder of combined form [25].
**Irritancy test**
Mark a surface of area of (1sq.cm) on the leftward dorsal surface. Fixed quantities of ready face packs were applied to the particular area and time was noted under observation. Reported for up to 24 hrs. for regular intervals if any sort of Irritancy, erythema, oedema, was patterned [26].This test was carried out in 3 males and 3 females volunteers and result was noted which is give in table no 6. The formulation presented no irritation, redness, edema not any Inflammation through irritancy studies. This formulation is harmless and safe to usage for skin.

## DISCUSSION

Herbal face packs are uses casually to enhance blood circulation, renew the muscles and help the skin to balance its elasticity and get rid of the dirt from skin pores. The front foot benefit of herbal cosmetics and goods are their non-poisonous nature, reduce the hypersensitive reactions and timely tested utility of many ingredients. The formulation was found similar, easily rinsing out and had very slightly alkaline pH that were well-matched with normal skin physiology. Angle of repose is typical related to resistance to the movement between particles or inter particulate friction. As per in terms and limit of Indian Pharmacopoeia the angle of repose the flow property has been classified. The outcomes of all these parameters shown that the dried powder of combined form possess good packing ability and decent flow properties. Therefore, it revealed better flow properties for formulation to attain clean, fresh and soft formulation.

## CONCLUSION

By using herbal preparations the existing skin conditions such as pimples, acne can be well treated. There are numerous herbal preparations as such herbal face pack, herbal face mask, face scrub act by several mechanism and supports to keep the skin healthy and nutrient. The existing formulations were formed by using several herbs which will help in recover fairness, diminish acne, by acting on face. The formulation was exposed to various evaluation parameters such as angle of repose, irritancy test and all the test was agreed by the formulation. Hence, polyherbal formulations have a widespread variety of choice in preventing skin problems. In the current effort, it is a great attempt to formulate the herbal face pack comprising naturally existing constituents like multani mitti, turmeric, sandalwood etc. It is suggested that the prepared formulation F1 was physico-chemically and microbiologically

stable and possessed characteristics of a standard cosmeceutical's formulation for skincare. Further optimization studies are required on this study to find the useful benefits of face packs on human use as cosmetic product.

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**Table 1 Formulation of herbal face pack (Quantity of sample for 100gm)**

| S.No. | Name of Ingredients | F1 | F2 | F3 |
|---|---|---|---|---|
| **1** | *Curcuma longa* powder | 7.5g | 10g | 8g |
| **2** | Multani mitti powder | 11g | 13g | 10g |
| **3** | *Santalum album* powder | 6g | 9g | 11g |
| **4** | *Citrus aurantium* powder | 8g | 8g | 7.5g |
| **5** | Green tea powder | 4.5g | 4.5g | 6g |
| **6** | Almond peel powder | 3g | 3g | 3g |

**Table 2: Organoleptic properties**

| S. No. | Formulation | Physical appearance | Odor | Taste | Texture |
|---|---|---|---|---|---|
| 1 | F1 | Brownish | Slight | Characteristic | Fine |
| 2 | F2 | Brownish | Slight | Characteristic | Fine |
| 3 | F3 | Brownish | Slight | Characteristic | Fine |

**Table 3: Physiochemical parameters**

| S.No. | Formulation | Moisture content (%) | Bulk density(g/ml) | Tapped density(g/ml) | Angle of repose (Degree) |
|---|---|---|---|---|---|
| 1 | F1 | 4.5 | 0.55 | 0.83 | 18.93 |
| 2 | F2 | 4.5 | 0.53 | 0.77 | 13.33 |
| 3 | F3 | 4.6 | 0.47 | 0.76 | 13.22 |

*Results were the average of three readings

**Table 4: General powder characteristic**

| S No. | Formulation | Total ash value | Acid insoluble ash | PH | Particle Size(μm) |
|---|---|---|---|---|---|
| 1 | F1 | 36.5 | 0.67 | 6.65 | 20-25 |
| 2 | F2 | 40 | 0.72 | 7.61 | 25-30 |
| 3 | F3 | 36.05 | 0.64 | 6.69 | 30-40 |

*Results were the average of three readings

**Table No. 5: Result of formulations for shinoda test (chemical test)**

| S. No. | Formulations | Observations | Result |
|---|---|---|---|
| 1 | F1 | Pink color obtained | Flavonoids present |
| 2 | F2 | Red color obtained | Flavonoids present |
| 3 | F3 | Pink color obtained | Flavonoids present |

**Table 6 Irritancy test****

| S. No. | Formulation (F1) | Irritation | Erythema | Edema |
|---|---|---|---|---|
| 1 | W1 | No Irritation | No Erythema | No Edema |
| 2 | W2 | No Irritation | No Erythema | No Edema |
| 3 | W3 | No Irritation | No Erythema | No Edema |
| 4 | M1 | No Irritation | No Erythema | No Edema |
| 5 | M2 | No Irritation | No Erythema | No Edema |
| 6 | M3 | No Irritation | No Erythema | No Edema |

*W= Women, M= Men

**Irritancy test was conducted on all 3 formulations but here results are shown for best suited formulation F1

**Nature of face after wash=** Soft and fresh, clean from dirt

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**RESEARCH ARTICLE**

# Comparative Analysis of Classifier Performance: A Study of Weka and Python Implementations across Various Machine Learning Models

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**ABSTRACT**

The primary goal of engineering colleges is to deliver high-quality education, a challenge intensified by the post-COVID transition from online to offline learning. This study aims to predict student performance to provide early interventions for at-risk students. The research has three main objectives: identifying factors influencing student performance, comparing the accuracy of various data mining and AI algorithms in Weka and Python, and determining the most effective classifier. Data from 689 students were analyzed using metrics such as accuracy, F1 score, recall, precision, ROC curve, and confusion matrix. Our findings indicate that background characteristics (family details, parent's education, income, employment status, and student gender), educational attributes (academic performance, attendance, and assignment scores), and psychological traits (mental and physical health) significantly impact performance. Among the classifiers tested, the Random Forest algorithm showed the 100% highest accuracy in both Weka and Python environments.

**Keywords:** Education, Algorithm, Artificial Intelligence, COVID.

## INTRODUCTION

The COVID-19 pandemic brought unprecedented changes to the educational landscape, necessitating a rapid shift to online learning. During this period, many students excelled in online examinations, often achieving perfect scores as schools and colleges adapted to virtual teaching methods. However, with the return to in-person classes, a decline in student performance on offline exams became evident, revealing gaps in the depth of learning that occurred in the online format. To address these challenges and enhance the effectiveness of student learning and performance

tracking, we propose leveraging data mining and artificial intelligence (AI) algorithms. Data mining involves extracting valuable insights from large datasets and has become an essential tool for data analysis across various industries[1]. In the context of education, particularly for first-year engineering and science students, these techniques can play a critical role in supporting academic achievement. Early academic performance is crucial as it significantly impacts students' cumulative GPA and overall educational trajectory[2]. Predicting student performance through mid-term and final exams can offer educators early insights, enabling proactive interventions for students at risk of poor academic outcomes[3]. Data mining techniques, especially classification and clustering, are vital in this predictive process. Classification sorts data into predefined groups, while clustering groups similar data sets based on shared characteristics. These methods allow educational institutions to analyze various attributes that influence student performance. Our research aims to gather and analyze the perspectives of key stakeholders—students, professors, teachers, directors, and industry professionals—through a comprehensive questionnaire distributed via Google Forms. By examining the responses, we aim to identify critical factors and their relationships in predicting student performance. This study seeks to answer the following questions:

1. How do various factors influence the student performance prediction?
2. Which Integrated Development Environment (IDE) yields the most accurate results, & what are the differences in accuracy among them?
3. Which classifier is the most effective in predicting student performance?

Through this research, we aim to provide valuable insights that can inform strategies to enhance student learning outcomes and overall academic success.

**Related Work**

Over the past decade, numerous studies have focused on predicting student performance using various data mining and AI algorithms. Here, we review key contributions in this area, highlighting the methodologies and accuracies reported.

- **V. Ramesh** *et al*.(2013)identified significant predictive variables and developed an algorithm for grading higher secondary students using decision trees. Their model achieved an accuracy of 85% in predicting student grades [4].
- Elakia *et al*. (2014) employed various decision tree algorithms, including ID3, C4.5, and CHAID, to suggest career options for high school students and monitor their behavior. Their findings indicated that the ID3 algorithm produced the most accurate results, achieving an accuracy range of 90-98% [5].
- C. Anuradha *et al*. (2015) explored the prediction of students' end-term results using multiple algorithms such as C4.5, KNN, Bayesian Network, OneR, and jRip. Among these, the jRip algorithm demonstrated the highest accuracy, with a 60% success rate in predicting end-term results [6].
- **Amjad Abu Saa(2016)** constructed a prediction model based on personal, family, and social factors using the Naive Bayes algorithm. This study highlighted the holistic approach to attributes and achieved an accuracy of 78% [7].
- Ihsan A. Abu Amra *et al*. (2017) utilized KNN and Naïve Bayes algorithms to assist the Ministry of Education in classifying students with degraded performance. The study revealed that the KNN algorithm provided the best results, achieving an accuracy of 93.6% [8].
- **Atta-Ur-Rahman** *et al*.(2018) developed a model focusing on student interest and feasibility using logistic regression. Their findings underscored the importance of student engagement, with the model achieving an accuracy of 80% [9].
- **Shubhangi Urkude and Kshitij Gupta (2019)**analyzed graduation rates and course completion rates using the Support Vector Machine (SVM) algorithm. The SVM model provided the best results, achieving an accuracy of 82% [10].
- **Vairachilai S** *et al*.**(2020)** identified various dependent and independent factors affecting student performance and applied multiple data mining algorithms, including Naive Bayes, Decision Trees, and K-Nearest Neighbors (KNN). The Naive Bayes algorithm emerged as the most effective, with an accuracy of 83% in predicting grades [3].

- **Raza Hasan** *et al*.**(2020)** explored various e-learning applications and applied different algorithms, including the genetic algorithm for feature selection. Their study found that the Random Forest algorithm yielded the best results, with an accuracy of 85% in predicting student performance [11].
- **J. Dhilipan** *et al*.**(2021)** aimed to recognize final grades and improve academic performance through the evaluation of four algorithms: Decision Trees, Naive Bayes, KNN, and Binomial Logistic Regression. Binomial logistic regression provided the best predictive accuracy at 79% [12].
- **Ahajjam Tarika, Haidar Aissab, and Farhaoui Yousef**(2021)evaluated various algorithms and identified the Random Forest algorithm as the superior method for predicting student performance, achieving an accuracy of 88% [13].
- Yahia Baashar *et al*. (2022) employed Artificial Neural Networks (ANN) to address theoretical gaps in predicting student performance, demonstrating the potential for improved accuracy and reliability in educational predictions [14].
- Nitin Ramrao Yadav *et al*. (2023) conducted an analysis of various machine learning and data mining algorithms, including ANN, SVM, Naïve Bayes (NB), Linear Regression (LR), and Decision Tree, to evaluate their effectiveness in predicting student performance. This comprehensive study provided insights into the strengths and weaknesses of each algorithm in the educational context [15].
- Zhaoyu Shou *et al*. (2024) utilized Long Short-Term Memory (LSTM) networks to predict student performance using data on learning behaviors, scores, and demographics. This approach considered the temporal interactions of these factors, providing a dynamic perspective on how they influence academic outcomes over time [16].

These studies collectively demonstrate the evolving landscape of student performance prediction. They highlight the effectiveness of various data mining algorithms and the importance of considering a wide range of attributes, from personal and social factors to student interests and e-learning application usage. The consistent identification of algorithms like Naive Bayes, SVM, and Random Forest, Artificial Intelligence, KNN, Decision Tree as top performers across different studies underscores their robustness and reliability in educational data mining. This literature survey provides a foundation for our research, which aims to further explore the predictive power of these algorithms and identify key factors influencing student performance. By analyzing stakeholder perspectives and employing advanced data mining techniques, we seek to enhance the accuracy and applicability of student performance prediction models.

**Attribute Selection**

Through an extensive literature review and brainstorming sessions with various stakeholders—including directors, department heads, professors, students, and engineers—we identified several factors influencing student performance. These factors were gathered through a comprehensive questionnaire designed to collect detailed information on students' personal, social, psychological, parental, school, and college backgrounds. The questionnaire, built using Google Forms, was distributed to and completed by 300 stakeholders. The factors identified through these discussions and responses are summarized in Table I. These attributes are categorized into three main sections background information, educational information, and psychological information.

## METHODOLOGY

Figure I illustrates the five stages involved in identifying the most accurate predictive results. Each section elaborates on the stages and observations.

**Data Collection**

After selecting relevant attributes, we gathered data from 689 B.Tech students from various streams at IP University. The dataset includes values corresponding to the chosen attributes, with student identities anonymized and labelled with unique codes.

**Data Pre-processing**

In this stage, we pre-processed the data to remove anomalies, missing entries, duplicates, and incorrect values. After cleansing the data, we processed it according to the criteria outlined in Table 1, classifying it into appropriate ranges.

**Statistical Analysis**

This stage involved identifying patterns, trends, and extracting useful information from the dataset. No outliers were detected during the analysis. We applied the Extra Tree Classifier to determine feature ranking. Table II displays the feature selection ranking, where higher values indicate more significant attributes. Figure II illustrates the feature ranking graphically. Additionally, we calculated the p-value for each element, which resulted in a significance level of 0.01.

**Model Implementation& Evaluation**

In our study, we implemented various machine learning models including Decision Tree (DT), Naive Bayes (NB), Logistic Regression (LR), Support Vector Machine (SVM), Random Forest, and K-Nearest Neighbors (KNN). For all models, we used a consistent split ratio for training and testing, with 70% of the data allocated for training and 30% for testing. We implemented the Decision Tree classifier using the entropy criterion for information gain, and allowed the tree to expand up to three levels. Random Forest classifier was implemented with 100 estimators, providing a robust ensemble learning approach to improve predictive accuracy. The SVM classifier was implemented with three different kernel types: linear, sigmoid, and polynomial. Each kernel was evaluated to determine the most effective for our dataset. We used the Gaussian Naive Bayes classifier, which assumes that the features follow a normal distribution. The KNN classifier was implemented with a specified number of neighbors (N estimators) to determine the optimal balance between bias and variance. In predictive modelling, various algorithms are used for predicting student performance. The most important and popular seven supervised algorithms implemented on the dataset are Decision Tree, Naive Bayes, Support vector Machine, Artificial Neural network, K-nearest neighbor, Random forest, and logistic Regression in Weka and Python. For evaluating the performance of these algorithms we generate the confusion matrix. Confusion matrix is a table that have two dimensions one is predicted and another one is actual. Both the dimension have four tuples i.e. true positive (TP) in this prediction model predict all the positive values of predicted and actual class are predicted correct or true, true negative (TN) in this prediction model predict all the negative values of predicted class and actual class are predicted negative, false positive (FP) in this prediction model predict all the negative values of actual and predicted class are predicted positive and false negative (FN) in this prediction model predict all the negative values of actual and predicted class are predicted positive and different metrics (accuracy, F-score, recall, precision, roc curve) of each algorithm that are described below:-

**Accuracy:** -it tests the data and gives the accurate predicted percentage of the predicted model.

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{FP} + \text{FN} + \text{TN}}$$

**Precision:** - It calculates the actual positive values from the overall positive values that are predicted by model.

$$\text{Precision} = \frac{TP}{TP+FP}$$

**Recall:**- It calculates positive values that is correctly calculated by prediction model.

$$\text{Recall} = \frac{TP}{TP+FN}$$

**F-score**:- it is calculated harmonic mean of precision and recall.1 represent the best value and 0 worst value.

$$\text{F1-score} = \frac{2*(\text{precision} \ *\text{recall})}{(\text{precision} \ +\text{recall})}$$

**Decision Tree**

Decision trees are a fundamental supervised learning algorithm. They operate using a tree-like structure where two key node types play a crucial role. Decision Nodes act as checkpoints, evaluating a specific feature of the data. Based on this valuation, the data is directed down different branches of the tree. Leaf Nodes represent the final outcome, indicating the predicted class or value for a given data point. The purity of each node, signifying how well-separated the data points are within a class, is measured using the Gini impurity metric. A perfectly pure node (Gini value of zero) contains only data points from a single class. Conversely, a higher Gini value indicates a more diverse mix of

classes within the node. Decision trees are powerful tools for various tasks. Their effectiveness can be assessed using metrics like precision and F-measure. The decision trees in Weka and Python are shown in Fig 3 and 4. Table III shows the confusion matrix of the decision tree in this result of f-measure and precision are higher for A-class in comparison to B-class. Table IV shows the model evaluation in Weka and Python. Weka gives 90% accuracy and Python gives 96% accuracy on the data set in comparison to Weka Python gives the best result. Fig V shows the ROC curve.

**Naive Bayes**

It is a probabilistic classifier based on Bayes' theorem, also known as Bayes' Rule or Bayes' Law. It calculates the probability of a hypothesis given prior knowledge and evidence. The theorem utilizes conditional probability to determine the posterior probability of a hypothesis given observed data. In this context, Table V presents the confusion matrix of a decision tree classifier, indicating higher f-measure and precision for class A compared to class B. Furthermore, Table VI illustrates the model evaluation results obtained using Weka and Python. Weka yielded an 84% accuracy rate, while Python achieved a 94% accuracy rate on the dataset. Python outperformed Weka, demonstrating superior performance in this evaluation. Fig VI shows the ROC curve

**Support Vector Machine**

This technique belongs to the realm of supervised machine learning. It aims to minimize error by establishing a hyperplane. SVM, or Support Vector Machine, combines three key components: support vectors, hyperplanes, and margins. Support vectors are data points situated close to the hyperplane, while the hyperplane itself segregates data points into distinct classes. Margins denote the separation between the hyperplanes that closely border different classes.

A radial basis function is employed in the kernel of this SVM approach. Interestingly, both Weka and Python yield identical accuracy rates of 84%. Table VII showcases the model evaluation conducted using the hold-out method in both software environments. Meanwhile, Table VIII displays the confusion matrix, revealing that the precision achieved in class B surpasses that of class A. Notably, among Naive Bayes and Decision Tree techniques, SVM stands out as the method attaining the highest precision specifically in class B.Fig VII shows the ROC curve

**K-nearest neighbor**

This algorithm operates within the domain of supervised learning. It retains a repository of all provided data and utilizes this dataset to categorize new data or records based on their resemblance to existing data points. This process ensures that new data is appropriately classified into the correct category. Initially, a user specifies the number of neighbors, denoted as 'k'. The algorithm then calculates the Euclidean distance between the new data point and each existing data point, selecting the 'k' nearest neighbors based on these distances. Subsequently, it tallies the data points belonging to each category and assigns the new data point to the category with the most similar values. The confusion matrix, depicted in Table IX, reveals that both precision and f-measure attain a value of 1, denoting perfect accuracy. Notably, this algorithm outperformed its Python counterpart when executed in Weka. Furthermore, Table X illustrates the model evaluation results in both Weka and Python, showcasing a remarkable 100% accuracy rate. Noteworthy, the parameter 'n_neighbors=19' is passed to the KNN classifier, indicating the number of neighbors considered during classification. Fig VIII shows the ROC curve

**Random Forest Classifier**

This classifier operates by constructing decision trees from different subsets of the dataset, subsequently aggregating predictions through a majority voting mechanism to determine the final classification. Table XII illustrates the model computation conducted by the classifier in both Weka and Python environments, showcasing identical and optimal results with a 100% accuracy rate. Meanwhile, Table XI displays the confusion matrix, wherein both classes A and B exhibit equivalent precision and F-measure values. Notably, the confusion matrices of both the KNN classifier and the random forest classifier yield identical outputs. Fig IX shows the ROC curve

**Logistic Regression**

This model predicts both probabilistic and categorical values. Probabilistic values are outputs ranging between 0 and 1, while categorical values are either 0 or 1, or represented as "no" and "yes". Table XIV presents the model evaluation results in both Weka and Python environments, where Weka achieves superior accuracy of 90% compared to Python. Table XIII depicts the confusion matrix, revealing identical precision and F-measure values for both classes.Fig X shows the ROC curve

**Artificial Neural Network**

The neural network, often referred to as a feed-forward network, comprises multiple layers through which input vectors traverse. Initially, the input vector enters the input layer, where a function is applied to it, and subsequently, this output is propagated through all subsequent layers. Each layer is assigned weights, which are iteratively adjusted during training to optimize the output of the problem towards the output layer. Table XV illustrates the confusion matrix, indicating that class B exhibits a higher precision value compared to class A. In Table XVI, Weka outperforms Python with an accuracy of 97%.Fig XI shows the ROC curve In Weka KNN and Random Forest classifiers predict the best 100% accuracy and MLP also predict the best 97% accuracy . In Python Random Forest predict the best 100% accuracy and Decision Tree, Naive Bayes, KNN also predict the best accuracy above 94% shown in Fig XII.

## CONCLUSION

The prediction of student performance is critical for optimizing educational processes for both students and teachers. Our study employed feature ranking through a dedicated algorithm, alongside descriptive statistics and p-value calculations for each feature to identify the most significant predictors. Objective 1 is the Influence of various factors on student performance prediction in this we categorized student data into three primary groups: background characteristics, educational attributes, and psychological traits. Background characteristics included variables such as family details (number of siblings, parents' education, income, and employment status) and student gender, which significantly influenced model performance, particularly for high-achieving students who benefited most from additional support. Educational attributes, including past academic performance (grades from 10th, 12th, and the first year of B.Tech), attendance, and assignment scores, were found to have the strongest impact on student outcomes predicted by the ensemble model. Psychological attributes, including mental and physical health, also played a critical role as better health correlated with higher academic achievement. By integrating these factors, our ensemble model effectively categorized students within a smart education system, enabling targeted support and improved learning outcomes.

Objective 2 is the comparison of integrated development environments (IDEs) for accuracy in this we evaluated which Integrated Development Environment (IDE) provided the most accurate results. The classifiers tested showed minimal differences in accuracy, precision, F-score, and recall values, with only a 2-3% variance, as illustrated in Figures XII. Objective 3: effectiveness of classifiers in predicting student performance in these various classifiers, including Decision Tree, Multi-Layer Perceptron (MLP), Naive Bayes, Logistic Regression, Support Vector Machine (SVM), Random Forest, and K-Nearest Neighbors (KNN), were utilized for prediction. In Python, the Random Forest algorithm achieved 100% accuracy. Similarly, in Weka, both Random Forest and KNN predicted outcomes with 100% accuracy. Consequently, the Random Forest classifier emerged as the most effective algorithm among all tested classifiers.

**Limitations and Future Work**

The primary limitation of this research is the relatively small dataset, consisting of only 689 students. Additionally, the study did not account for factors such as students' social interactions, academic engagement, and interpersonal skills, which can also influence academic performance. Future research should aim to incorporate a larger dataset, include additional attributes or factors, and apply more algorithms to achieve the highest possible accuracy.

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**Table 1 Factors Description**

| Factor | Description |
|---|---|
| **Background Attribute** | |
| Gender | Student identifies as male, female, or non-binary. |
| Mother's Occupation | Mother's professional field (e.g., teacher, healthcare, civil service, homemaker, other). |
| Father's Occupation | Father's professional field (e.g., teacher, healthcare, civil service, homemaker, other). |
| Number of Siblings | Number of siblings, which may impact student focus and available resources. |
| Mother's Education Level | Mother's highest level of education, potentially influencing the student's educational aspirations. |
| Father's Education Level | Father's highest level of education, potentially influencing the student's educational support. |
| Family Income | Annual family income, affects the availability of educational resources and opportunities. |
| Caste Category (if applicable) | Student's caste classification (SC, ST, General, OBC) for potential affirmative action considerations. |
| **Educational Background Attribute** | |
| B.Tech 1st Year Marks | Average marks obtained in the first year of B.Tech, across semesters 1 and 2. |
| 10th Standard Marks | Percentage score in high school exams, indicative of foundational academic performance. |
| 12th Standard Marks | Percentage score in higher secondary school exams, reflecting pre-university academic achievement. |
| B.Tech Admission Mode | Mode of admission into the B.Tech program (entrance exam or management quota). |
| Scholarship Status | Indicates whether the student receives a scholarship from the university, potentially reducing financial burden. |
| Assignment Performance | Contribution of assignments to the overall internal marks, reflecting consistency in academic tasks. |
| Class Attendance | Frequency of the student's presence in lectures and labs, indicative of engagement with coursework. |
| **Psychological Attributes** | |
| Primary Language | Student's preferred language for communication (English, Hindi, etc.), which may affect comprehension and participation. |
| Overall Health Status | General health condition, potentially impacting the student's ability to perform academically. |
| Parental Relationship Status | Status of parents' relationship (living together or separated), which may influence the student's emotional well-being. |
| Commuting Time | Time spent traveling between home and college, potentially affecting available study time and overall energy levels. |

**Table 2 Feature Importance of each attribute**

| Features | Features Importance |
|---|---|
| feature 0 | (0.106895) |
| feature 1 | (0.169679) |
| feature 2 | (0.101148) |
| feature 3 | (0.039577) |
| feature 4 | (0.037702) |
| feature 5 | (0.038873) |
| feature 6 | (0.035574) |
| feature 7 | (0.039569) |
| feature 8 | (0.038789) |
| feature 9 | (0.039869) |

| | |
|---|---|
| feature 10 | (0.039171) |
| feature 11 | (0.039274) |
| feature 12 | (0.038094) |
| feature 13 | (0.039570) |
| feature 14 | (0.039626) |
| feature 15 | (0.038963) |
| feature 16 | (0.037756) |
| feature 17 | (0.040460) |
| feature 18 | (0.039411) |

**Table 3 Confusion matrix of Decision tree**

| **Actual/Predicted** | **A** | **B** | **Precision** |
|---|---|---|---|
| **A** | 61 | 6 | .93 |
| **B** | 4 | 29 | .82 |
| **F-measure** | .92 | .85 | |

**Table 4 Model Evaluation of Decision Tree**

| | Weka | Python |
|---|---|---|
| Accuracy | 90% | 96% |
| F-score | .90 | .95 |
| Precision | .90 | .95 |
| Recall | .90 | .95 |
| ROC | .92 | .95 |

**Table 5 Confusion matrix of Naive Bayes**

| **Actual/Predicted** | **A** | **B** | **Precision** |
|---|---|---|---|
| **A** | 62 | 5 | .86 |
| **B** | 10 | 23 | .82 |
| **F-measure** | .89 | .75 | |

**Table 6 Model Evaluation of Naïve Bayes**

| | Weka | Python |
|---|---|---|
| Accuracy | 84% | 94% |
| F-score | .84 | .95 |
| Precision | .84 | .95 |
| Recall | .84 | .95 |
| ROC | .90 | .95 |

**Table 7 Confusion Matrix in SVM**

| **Actual/Predicted** | **A** | **B** | **Precision** |
|---|---|---|---|
| **A** | 67 | 0 | .80 |
| **B** | 16 | 17 | 1.0 |
| **F-measure** | .893 | .68 | |

**Table 8 Model Evaluation of SVM in Weka and Python.**

| | Weka | Python |
|---|---|---|
| Accuracy | 84% | 84% |
| F-score | .82 | .82 |
| Precision | .87 | .84 |
| Recall | .84 | .84 |
| ROC | .75 | .8 |

**Table 9 Confusion Matrix of KNN.**

| **Actual/Predicted** | **A** | **B** | **Precision** |
|---|---|---|---|
| **A** | 67 | 0 | 1.0 |
| **B** | 0 | 33 | 1.0 |
| **F-measure** | 1.0 | 1.0 | |

**Table10 Model Evaluation of KNN in Weka and Python.**

| | Weka | Python |
|---|---|---|
| Accuracy | 100% | 95% |
| F-score | 1 | 0.93 |
| Precision | 1 | 0.90 |
| Recall | 1 | 0.90 |
| ROC | 1 | .92 |

**Table 11 Confusion Matrix of Random Forest Classifier.**

| **Actual/Predicted** | **A** | **B** | **Precision** |
|---|---|---|---|
| **A** | 67 | 0 | 1.0 |
| **B** | 0 | 33 | 1.0 |
| **F-measure** | 1.0 | 1.0 | |

**Table 12 Model Evaluation of Random Forest Classifier in Weka and Python**

| | Weka | Python |
|---|---|---|
| Accuracy | 100% | 100% |
| F-score | 1 | 1 |
| Precision | 1 | 1 |
| Recall | 1 | 1 |
| ROC | 1 | 1 |

**Table 13 Confusion Matrix of Logistic Regression.**

| **Actual/Predicted** | **A** | **B** | **Precision** |
|---|---|---|---|
| **A** | 62 | 5 | .925 |
| **B** | 5 | 28 | .84 |
| **F-measure** | .925 | .84 | |

**Table 14 Model Evaluation of Logistic Regression. in Weka and Python.**

| | Weka | Python |
|---|---|---|
| Accuracy | 90% | 80% |
| F-score | 0.90 | 0.8 |
| Precision | 0.90 | 0.82 |
| Recall | 0.90 | 0.83 |
| ROC | 0.92 | 0.72 |

**Table 15 Confusion Matrix of Multilayer Perceptron Network.**

| Actual/Predicted | A | B | Precision |
|---|---|---|---|
| **A** | 67 | 0 | .95 |
| **B** | 3 | 30 | 1 |
| **F-measure** | .97 | .95 | |

**Table 16 Model Evaluation of MLP in Weka and Python**

| | Weka | Python |
|---|---|---|
| Accuracy | 97% | 90% |
| F-score | .97 | .90 |
| Precision | .97 | .90 |
| Recall | .97 | .90 |
| ROC | .92 | .90 |

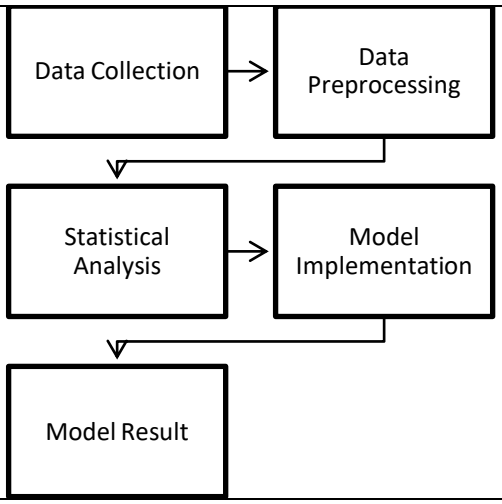



**Fig 1 Methodology used in Research**

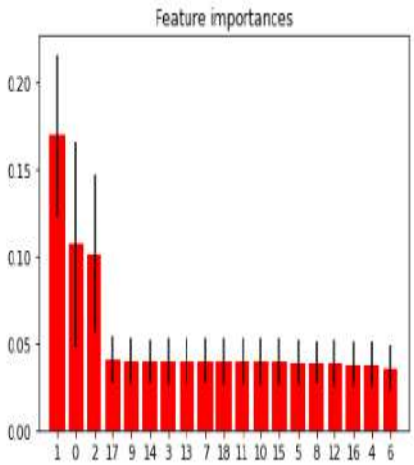



**Fig 2 Feature ranking graph**

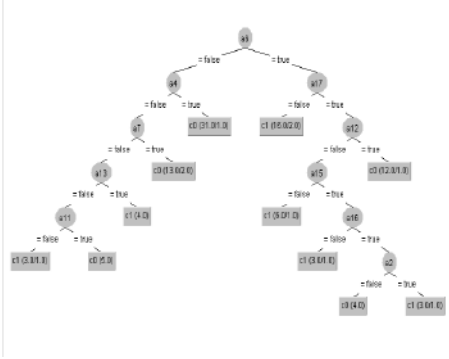


**Fig 3 Decision Tree in Weka**

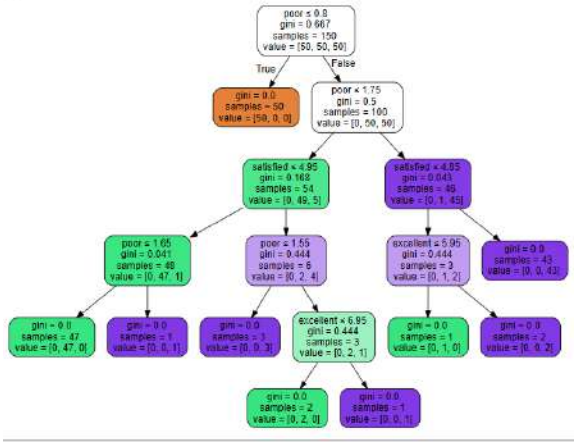


**Fig 4 Decision Tree in Python.**

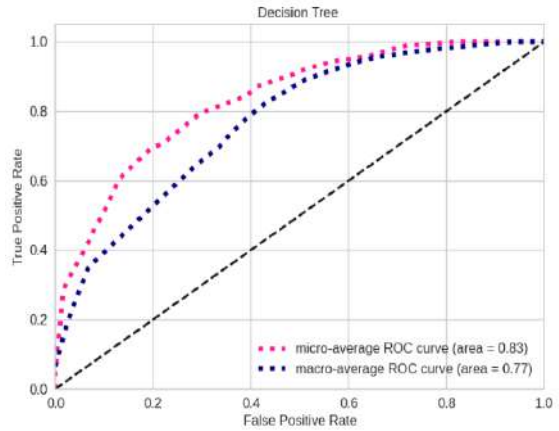
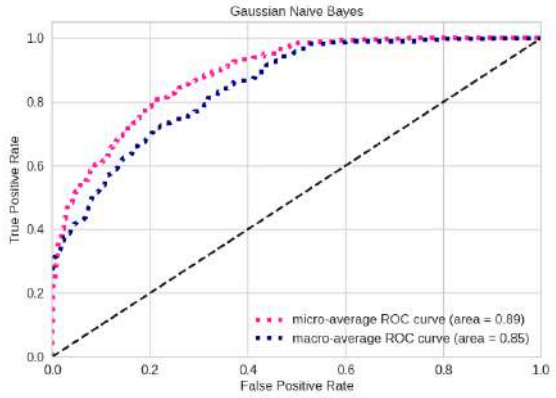



**Fig 5 ROC Curve of Decision Tree**




**.Fig 6 ROC Curve of Naïve Bayes**

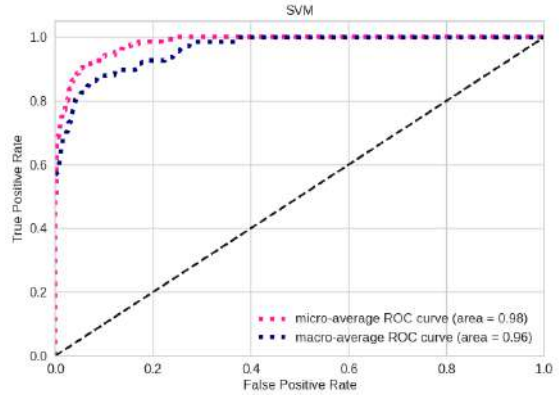



**Fig 7 ROC Curve of SVM**

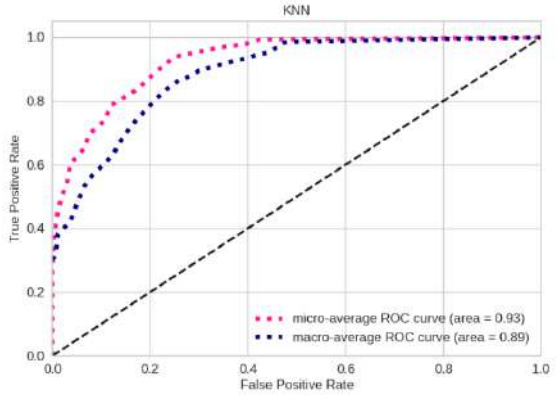



**Fig 8 ROC curve of KNN**

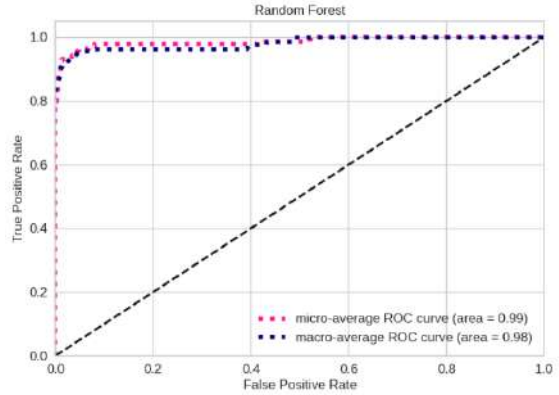



**Fig 9 ROC curve of Random Forest**

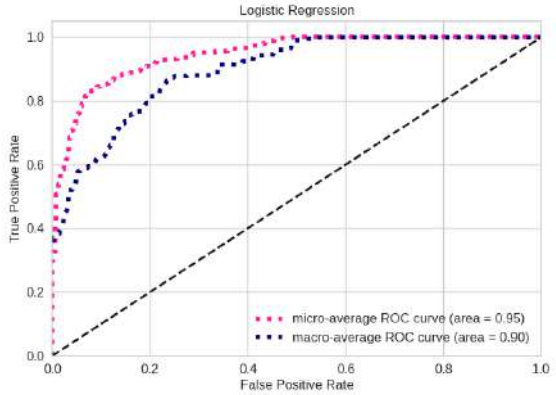



**Fig 10 ROC Curve of Logistic Regression**

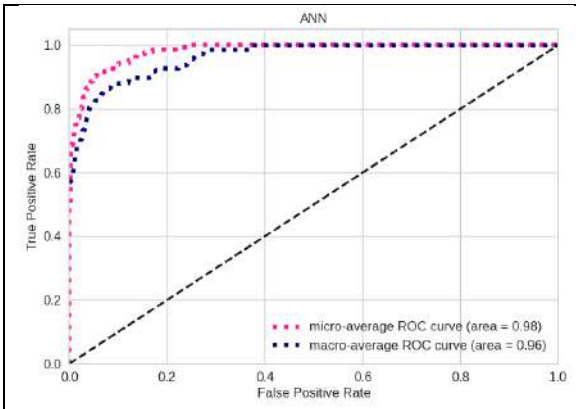



**Fig 11 ROC curve of ANN**

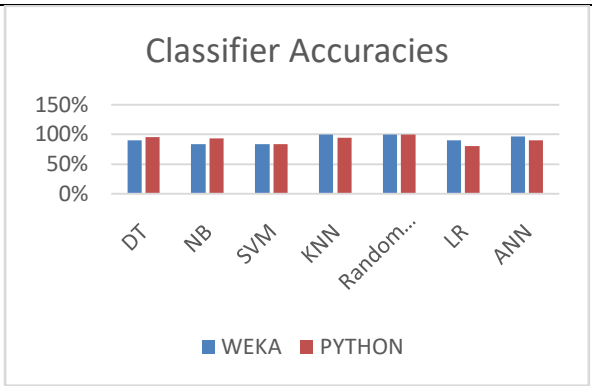



**Fig 12 Accuracy comparison between Weka and Python**

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**RESEARCH ARTICLE**

# GMOS? - Superficial Sustainers? Or Capital Combating Crusaders?

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## ABSTRACT

Life has always been evocative, if its naturally sustained not artificially altered: alterations are justifiable in dire circumstances of do or die: yet not desirable in all dare to do settings where existential necessities are manipulated for contextual conveniences, where man attributes utmost weight age to self and egotistical motives, negotiating with the collective harmony and well-being of the web of life. Humans have lost the gist of eloquent existence hence mislaid the mirth of life in the duel of one outweighing the other, favouring world orders founded on control and confinement giving way to confusion and chaos yielding an speace or sans happiness to the revolutionary or the revolutionised: neither the master nor the slave reap real reimbursements. Most of the revival happening through the propagation of science and technology are benefitting generally the capital collectors. One such capital gaining strategy that might have provoked the initiation of the Gain Manipulating [Gambling] Order: occurs to be GMO. This paper investigates the initiation, survival cum credibility of GMO, provoking the public to rationalize, scrutinize and conceptualize its utility and propagation. Many might marvel about the global role played by GMOs in our ecosphere escalating significantly the dialogs that abound GMOs, in all social media platforms over the past few years: yet the ambiguity surrounding GMO still lingering on in all the developing countries other than the progressive countries around the world, which promotes cognizance hence that have limits or total prohibition for manufacture plus dispensation of the same, then majority have been undoubtedly exposed to them rather consumed it inadvertently as their rewards are projected than their outlays. Artificiality needs more propagation than naturally prevailing support systems, as simplicity seldom sells in this era of propaganda!

**Keywords:** GMO Superficiality Biotechnology Artificiality Manipulation

# INTRODUCTION

**Grasping the Genetics of GMO**

Genetically modified organism (GMO)as the world comprehends it, or it's draftsmen designed it, has its genetic material transformed out of inquisitiveness targeting superficial benefits via genetic engineering methods: targeting superficiality in terms of benefit itself raises a concern activating artificial advance in the name of evolution: its steered in anticipations of either obtaining favourable traits, eliminating unfavourable qualities, or simply gene manipulation the precise interpretation, its makeup in heritable engineering differs, with the utmost mutual existence: has been reformed not by obvious breeding or usual recombination. An extensive diversity of creatures are apparently hereditarily revised (GM), from beasts to vegetation plus microbes, transmuting the inheritable factors of the same species, across species altering the transgenics athwart domains. Novel genes are familiarised, there by attracting, altering and knocking out endogeneity: a beneficial game authored by benefactors for beneficiaries. Superficially stupendous strides are embraced like quick fixes, quaking the clarity of creation.Never to be muddled with outdated breeding observes, here diverse breeds of the identical species are united to yield favourable individualities. Such tentative groupings of genes doesn't emerge naturally: hence unnatural. Purpose of exclusivity is undeniably debilitated to the extent of conception of initial indistinct imbalances further drawing the situation incomprehensible hence riotous.

Riotous risks rattle right to existence of each entity entailing the upkeep of ease of earth. Generating an intricate inherently adapted being is undoubtedly a multi-step progression. Genetic applied scientist details isolation of the genetic factor that's demanded to be supplemented into the host organism and associate it with other inherited fundamentals. Innumerable Procedures are accessible for implanting the secluded gene into the host genome. Current Progressions By means of genome editing procedures, especially CRISPR, have concluded the manufacture of GMOs much simpler. Forerunners in generating the primary GMO in 1973,Herbert Boyer and Stanley Cohen Positions to be the innovators of a bacterium buoyant to the antibiotic kanamycin. Rudolf Jaenisch shaped the preliminary GM animal, a mouse, in 1974 , followed by the foremost shrub in 1983.Precisely After fourteen years, the year 1994, observed the problem of Flavr Savr tomato, the foremost marketed GM food. The primary GM animal that happens to be marketed was the GloFish (2003) and the foremost GM animal to be permitted for sustenance happens to be the Aqu Advantage Salmon in 2015.The mainstream GMOs are concocted branding them too lenient to bulky birds of insecticides and weed killers, like Roundup, a biochemical enricher designed by the establishment Monsanto. Glyphosate a possible human carcinogen, was found in Monsanto's Roundup, that was avowed by the World Health Organization in May 2015. Commonplace Weeds Seldom survive while bare to these punitive substances, points to prowl on GM corn's unnatural genetic makeup premeditated to subsist when administered. GMO yields are held accountable aimed at the advent of "super weeds" and "super microbes" that could lone be slayed by the deadliest lethal contagion alike 2,4-D (a key component in Agent Orange). The agriculturalists pretentious by resilient pests should be advised to return to previous plus complex lethal elements should essentially gather more labour or more rigorous ploughing, that outshine the assured reimbursements of GMO expertise.

**Kenyan Epitomes on GMO effectuality/ ineffectuality**

This pursuit would vouch to remark about one such dwelling that apparently got influenced by GMOs is none other than Kenya: Lately Kenya fixes to commodify heritable reformed crops, gaining confrontation from few farmers and operation clusters, that interrogates their security. Allegations of farmer Eva Wanjiru, points to the abnormalities of artificially engineered edibles, floating her apprehension over the administration's current reversion of a decade ban on the inherently modified crops. Ms Wanjiru executed organic agriculture for years, refusing to employ insecticides or fusion kernel in her farm, trusts that there is dearth of much satisfactory signal to verify that yields fashioned through biotechnology will be backing the nation battle sustenance diffidence as utmost farmers grumble about pests and ailments, who promote GMOs. Scarcity of rain, compel them to whine about the yield's meagre coping in the farm dispersing qualms about farmer's absolute reliance on corporations that trade the GMOs, and the threat of their command of the marketplace to the hindrance of commonplace Kenyan farmers. Eventuality of pronouncement calls

to initiate thought processes guiding to comprehend the malign intentions of capture for capital gains. Such quick fixes as solutions to problems have so far served as only superficial solutions.

Consenting these companies to oversee; affects the upkeeps of the agriculturalists, in Kenya, manufacturing maize yearly reports, Claire Nasike, conservational researcher at Greenpeace Africa exposes the crux of the capture: as withdrawing the GMO prohibition exposes agriculturalists to draconian intellectual property regulations linked to copyright detained by GMO corporations as the GM seed is patented and this might lead to mooring the agronomists deprived of their information into intellectual possession rows, clarified Ms Nasike .Yet Dr Stephen Mugo's argument refutes the idea that Kenya will be under the kindness of. director of the Centre for Resilient Agriculture for Africa, moreoverit's unbelievable assumption subsequently Kenya has the capacity to grow GM harvests. Maximum Corporations lease the expertise which they use create novel genes, Apparent exploitation of natural resources in the name of scientific revolution: what nature nurtures and serves freely for all is patented for profit: a sort of hypocritic hypnotism that holds the nerve paralysing the simple thought processes of ordinary man with the aid of heightened hyped up exaggerative inventions that confuse more than infuse, quake more than comfort bringing in slow devastation rather than sincere conservation. A study steered via a non-governmental body, Route to Food Initiative, preceding year presented that more than half of Kenyans unwelcome GMOs.Kenya is one of countries in the mainland favouring GMO use, presently approved for agronomy in 70 countries throughout the world. Yet The above cited securities will not suffice in resounding dubious community dwellers such as Ms Wanjiru to cultivate, or consume, GMO crops for the reason of their apprehensions about the security and misgivings over the prediction of financial reimbursements. Why such security apprehensions and fiscal benefit qualms arise even after solid promotions and publicity. Rise of scepticism has constantly been measured with the wake of artificiality and greed.

**Carbon Clutching by Progressive Photosynthesis Procedures for Taming Temperature Transformation**

Climate variation prevails all over the world, the prevalent traditions of farming hardly serve the purpose. The demand is about somegrander, more miscellaneous variety of crops that perform even in adverse circumstances. Presently, asper the United Nations' Food and Agriculture Organization, mere 15 crops make up 90% of our liveliness intake, familiarizing, folks who try evolving food crops that might prosper in our varying world, to benefit fraught farmers in parched areas. Much research has been taken forward in the course of tackling adversity in the desired direction, yet in haste to ease of tackling testing times, man often apparently is forced to support the unnatural further destabilizing the natural course of life events. Sangita Myska interacts with the co-initiator of Living Carbon, Patrick Mellor, who facilitated the advance of the world's first GM trees precisely planned to grab more carbon from the atmosphere, with a biotechnology developed to improve photosynthesis in poplar trees, permitting them to breed quicker and also fight decay for extended periods, yet how far that is going to contribute to the harmonious persistence of the web of life, only test of time can reveal as individual endeavours entertain or are limited to personal gains and accomplishments. Living Carbon anticipates to employ these trees to influence the level of carbon draw down required to uphold an ideal level of carbon dioxide in the atmosphere. Yet alas! The question initiating debate here is: Are sooner growing trees the evolutionary hack we've been looking for? Some favour the GM harvests, hence found them as a technique to resolve the biosphere's sustenance catastrophe and demanded they could formulate "the biggest revolution of a lifetime". Opposition Assumed that hereditary alteration went contrary to the regulations of nature, and would create the alleged Frankenstein foods.

The year 1994 witnessed FlavrSavr tomato to be the initial hereditarily altered harvest appropriating itself for business in the US, ultimately planning its track to British hypermarket shelves in the tomato puree formula, but once campaigners disclosed this, they rejected sellers from supplying it and other foodstuffs consequential from GM crops. Over a succession of months, campaigner shattered the fields of Government supported trials of GM maize and soya, Prince Charles went one-on-one with the pro-GM founding, enabling the hypermarket chains mandatory in reassuring the public that they were devoid of GM "polluted" goods. The key benefit of GM food is that crop harvests turn out to be more dependable and fruitful, permitting extra individuals to be nourished, rather there are arguments about world manufacturing more calories than it is mandatory for all the people on the planet to be hale

and hearty. Individual discretion should seldom be permitted to be overridden by artificial intelligence, as man has not been successful in substituting anything natural with artificial. Hence artificiality breeds limitations, irrationality and ill-health. Validating the mentioned in authenticity through research will lead one to study about pros and cons of genetically modified organisms. GMOs are not deprived of drawbacks. Nonetheless the absence of decisive links, Brown University resolved that vicissitudes to nourishments on a heritable level syndicate proteins that people hardly consume, swell the probabilities of a hypersensitive response stirring, escalating the rates of nutrition allergies in children since 1999.

**Advantages of GMO**

Recompenses of GMO" s needs some reference though drawbacks may outweigh rewards. Primary pounce of compensation arises as food supplies turn out to be foreseeable, crop harvests become more dependable and fruitful, letting more people to be nourished, notwithstanding the prerogative of feeding nutritious food than mere quantity. Dietary Plus content could be enhanced cum altered, provisioning a deeper dietary outline than what preceding peers were able to relish, means future generations could progress in receiving the equivalent nourishment from lesser levels of nutrition intake. Will all these expectations could gain ground and be real or are these prospects publicity gimmicks for capital gains. Global vitamin deficiencies are condensed by the utilization of high levels of vitamin A in Genetically modified rice, records the UN Food & Agricultural Department. GM foods can have extended ledge life that can abstain the aid of additives to sustain diet freshness enhancing the accepted potentials of the food itself. Conservational Nourishment, upholds the risk of certain preservatives that are linked with a higher carcinogen, heart disease, and allergy risk.

Prowess of producing some proteins and inoculations, end to end with added pharmacological properties, are considered to be medical benefits from GMO crops, a practice subscribing inexpensive means of refining individual health: by eating dinner receiving a tetanus promoter as an alternative of receiving an arm inoculation – that's assumed to be the outcome of this expertise, generating diets that are extra tempting to consume. Colours can be either transformed or enhanced with GM foods to enable them more attractive to eat. Spoon University notify those profounder colours in diet deviates the perception of what is being consumed. Resonant red colours make diet seem honeyed, even if it is not: as livelier foods are connected with enhanced nourishment and flavours. Moreover, GM foods are easier to transport as they are known for their extended shelf life, inviting the opportunity to eliminate food waste, increasing the possibility of hunger reduction and elimination in developing countries. Meticulous usage of herbicides and pesticides curbs the threats on croplands that's bound to eventually turn the soil ineffectual, agriculturalists growing genetically modified foods seldom use these foodstuffs as frequently as agronomists using old-style bidding procedures, permitting the earth to recuperate its nutrient foundation over time. In spite of the heritable confrontation being in the plant itself, the grow ermanages to accomplish a foreseeable produce at the similar time.

**Downsides of GM Foods?**

Man-made mechanisms mediate, mirror, medicative benefits but all those perks personify a greater scale of disruptive disorganisation in the normal/ mundane events that are called to catalyse the consumerist culture. GMO yields may cause antibiotic resistance. It is high time to park self and scrutinize whether the cons outweigh the pros, the investigation done in Iowa State University research estimates that harvests adapted to contain antibiotics and other items that slay microorganisms and pests, by diminishing the effectiveness of an antibiotic or additional medicine when it is obligatory in the old-style logic. Some amount of the antibiotic dash in GMO" s when consumed, is affected by a treatment antibiotic due to build resistance to it, that causes cure of illness difficult. A greater legal liability, is generated on the farmers growing GMO, due to the fact that harvests that are heritably adapter is guaranteed to generate kernels that are hereditarily revised. Moreover, the likelihood of cross-pollination between GMO crops and non-GMO crops are followed during definite agricultural observes, since many of the harvests and kernels that produce GMO crops are unproved, exposing the peasant to a complex level of lawful accountability. Agriculturalists Who aren't involved in the above process could also go through obligations for leasing seeds and authorizing their utility in other fields or allowing cross-fertilization to occur.

Genes are driven into dissimilar plant sorts further crop's part grounds with supplementary plants, together with weeds. Genetic relocations are notorious to transpire. Research about the ordeal of transfer of DNA segment from a herbicide-resilient harvest permeating into the weeds it is premeditated to slaughter? Communications at the cellular level possibly will produce unanticipated snags to forthcoming harvest development where even the reimbursements of GM foods might not outdo the complications rooted by them. In an effort to defend their profits over 50% of seed manufacturers forbid any self-governing study on the concluding yields as some GM foods may pose a carcinogen contact hazard. Multiple paper publications and retractions on the topic, exposed that harvests lenient to profitable pesticides significantly amplified the danger of cancer advance in rats. Nonetheless restricted, this evidence has been extensively spread creating the imprint that all GMO foods are hypothetically perilous.

Ananalysis directed by researchers scrutinise the conducts and mechanisms that aids extraneous DNA helps subsist through absorption and infiltrate into the body cells of the user. Scientists are predominantly concerned in reviewing the gastrointestinal tract being the hot plug for parallel gene transmission of GM yield DNA into gut-bacteria. Pointing the consequence of the mentioned allocation can theoretically cause dysbacteriosis and bad-health, as well as successive transmutations. Researchers pondering on the incongruities that ascend about the issue of the occurrence of DNA of disbursed foodstuffs in the blood and organs of their customers further affirming the fiasco to illustrate their consequences on the human body, nonetheless they are also deliberating the likelihood of mixing genomes and the appearance of extraneous DNA in consumer's tissues. In supposition, scientists contemplate the penetration potential of micro-RNA of GM plant diet into the human body cells.

The recompenses and drawbacks of genetically modified foods can spur a nasty deliberation. The benefits of provisioning the ecosphere with improved diet access, should not be familiarized at the outlay of individual well-being. GMO foods must essentially be branded in EU and appeals in the US are in quest of an identical scenario. We warrant to identify what we're consuming and how that diet is made. The dispute around GMO is enormous and intense on either side. Notwithstanding one of the chief contemplations when at variance with the usage of GMO goods is the environmental devastation potential. Prioritizing the ecological hazards to contemplate in regard to GMOs: Chiefly, it is imperative to comprehend what a GMO is precisely, where WHO outlines them as entities whose genetic material has been transformed in an artificial way. GM floras are typically formed for bug resistance, virus resistance, or weed killer tolerance: approval of such unnatural alterations bring in some hypothetically challenging conservation challenges.

**Environmental Hazards of GMO!**

Potential human hazards were under the public scanner, whereas environmental threats can pose a coercion to the human environment bond stability supporting the steadiness of the web of life. Primarily, noxiousness is an enormous problem neighbouring biochemical insecticides and weed killers, employed typically with GMOs, in calculation with the harmfulness integral to these plants. Toxicity of GMOs to non-target organisms is quite evident, taking the visible communication illustrations of bees and butterflies, bees are immensely imperative in pollinating many diet harvests but are regrettably enormously threatened by contemporary agrarian practices, such as GM crops. Birds are equally at peril from insecticides and toil as organic regulators, negotiators and pollinators, alike bees. Additionally, the long-standing effects of GMOs aren't firm but uncertain, conditioning pests to familiarize to insecticides and weedkillers, in calculation to the DNA variations in GM plants enabling them ¨resilient. ¨Effectively this deciphers the doubt in authenticity of their efficacy, yet their lethal legacies will persist.

Collective paraphernalia of goods such as GMOs should be taken into contemplation. Recommendation Too Endorses that minor hereditary variations in plants fabricating even bigger biological swings, divulging the latent for GMOs to turn out to be tenacious and weedy in agronomic environments, since such has been altered to be resilient to some contemporary farming procedures, the hostility of GMO in natural settings seldom transpire logically, where the impossibility of novel, man-altered vegetation to turn out to be aggressive species is subtle, usual bionetworks could be ruled out. In conclusion, biodiversity, the critical component of all ecologies assimilating to the sustenance of all species, is put to danger by GMOs. The plantation of GM crops, commonly in a monocrop manner,

countless custom seeds are seldom used, upsetting the natural course of nature yet promoting the nature of GMOs through fewer wildflowers and, therefore, not as much of sap for pollinators. Contaminants Let unregulated into the earth through the plants´ paths mean threatened mud bacteria, that are essential to conserve vigorous soil for plants growth without the use of biochemical enrichers. Nutrients are rarely reimbursed to the earth in mono harvests and from GMO diets, as poisonous residues are left in the soil, making it parched and annulled of all nutrients, mostly essential to the budding procedure, further creating a sequence of reliance on GMO kernels and biochemical enrichers, insecticides, and weedkillers in then fashioned in order to produce a solitary harvest. Adding on to earth matters, the irrigation measures that are employed for organic growth of GM foods, transmits innumerable complications into aquatic springs and the air, divulging diverse microorganisms, bugs, and beasts to identical difficulties.

Many of such influences must be booked into contemplation in the bigger representation; GMO´s DNA may terminate in the earth, manure, animal feedstuff and by-products, and further active creatures, from bugs to biggerbothers. Bees transfer insecticides, weedkillers, and genetic material through the air into the atmosphere. A plant partnering in the agronomic environment, islucid to accept it to be a fragment of a grander bionetwork, apparently the threat of conservation harm done by GMOs is much greater than merely hypothetically impairing our healthiness.Apart from conservation matters, GMOs have been the topic of communal and moral deliberations as well. rooting a complex range of significances. Being conversant about the diet we are consuming and the methods contemporary agronomic techniques are upsetting the atmosphere is one operative technique of intentional interaction with the normal world.

**Influence, GMOs Inflicts on Society and the Setting**

In incongruity in what way mainstream opinions are fashioned, GMOs have essentially triggered the use of weedkillers and insecticides to upsurge pointedly, enabling the lingering of more biochemical deposit on yields for customers to consume, disclosing study around GMOs contradictory; nevertheless, there is a swelling body of indication linking GMOs with ecological damage, well-being problems, and even defilements of customers' and agriculturalists civil liberties. Many health destabilising diseases and disabilities like Autism, gluten intolerance, birth defects, besides several additional shocking wellbeing matters are all allied to Monsanto's Round Up. Some agronomists are satisfied with GMO diversities, others are dissatisfied, thus discovering wide-ranging outcomes or confronting new snags in the extremely intense and business conquered seed sector, such challenging drift disturb all agronomists, whether they favour GMO seeds or not. Jeopardy of Cross adulteration with GMO seeds is communal though the farmer refrains from using GMO crops. International Journal of Food Contamination observed innumerable cases of GMO adulteration between 1997 to 2013 in 63 nations, exposing agronomists to severe, dire penalties. Adulteration Catering the role of a catalytic agent for melodramatic financial fatalities for growers facing refusal from exportation bazaars that prohibit GMOs. Biological Agriculturalists suffering adulteration could drop their biological authorization and the premium for their biological yield. Further when request for non-GMO foodstuffs wells, agriculturalists are looking for prospects to spread into no-GMO marketplaces that recompense advanced charges, nevertheless, the incapability of businesses to suitably detach GMOs from conservative variation slingers to lurk agronomists' selections.

The lasting well-being paraphernalia of GMOs, mutually on the globe and society are mysterious. Adding on, approximately all scholarships that entitled to be innocuous are sponsored by the only bioengineering formations that yield from GMO auctions. Biotechnology Business Interprets GM yields as an advantage to our ecosphere for the reason that they permit agriculturalists to create advance harvest yields with scarce contributions. The kernel marketplaces have been inundated with GM soybean, corn, and cotton seeds with two major traits: insecticide expression and weed killer resistance, by big seed companies So, what does this contribute to? Essentially equipping the vegetation with the aptitude to subsist with detrimental microbe killers and weedkillers with the deadly characteristic of Bacillus thuringiens is, or Bt. Bt is associated to a class of microorganisms that reasons diet intoxication and anthrax, also slays only very exact class of pests. Numerous Biological Agronomists have used Bt for over 50 years as an insecticide to regulate bugs. Here and now, genes from Bt are instrumental in amending

vegetation to yield the Bt toxins and slay bugs that try to plague them without the application of exterior spraying. Researcher Charles Benbrook at Washington State University verified the upsurge of GMOs in the US has led to an intensification in the use of lethal biochemical involvements. Benbrook exposed that while the Bt feature has permitted agriculturalists to spray intensely smaller levels of pesticides, that outcome has been further overshadowed by the incursion of weedkillers unconstrained by Monsanto's Roundup Ready technology, as weeds have swiftly modified confrontation to recurrent dosages of Monsanto's Roundup weed killer.

**Do They Yield More Food? - Consolidation of Need Vs Greed!**

Investigatory logic around all these scientific technological evolutionary discoveries lingers around the ever-escalating unaccountable population and hitches associated with catering to such mounting statistics. So here, nowadays the quaking enquiry is, do GMOs harvest extra nutrition? A lately published article by University of Wisconsin has annulled the dispute that GMO agriculture profits additional food. The investigator equation harvest yields from numerous diversities of blend corn, some hereditarily altered and some not, between 1990 and 2010:nevertheless, some hereditarily adapted diversities generated insignificant advances, others did not. Myriad even exhibited inferior harvests than non-genetically modified equals. Investigate Information Determined the startling not so strappingly optimistic transgenic profit effects. Both the Bt characteristic for corn rootworm and glyphosate-tolerant prompted harvests to drip. Calculation, exposed indication of what is recognized as "yield drag" — the clue that deploying the genome of a shrub diversity sources inadvertent fluctuations in the mode it nurtures, triggering it to be not as much prolific.

However accurate this may seem to be, it is remote from producing advanced harvest yields with scarc involvements. The clarity of GMO seeds having compensations over conservative kernels when it derives to hazard justification, exceed the reimbursements presented by biological farming. Consequently, profound study is required to discover the new constituents of biological compost for snowballing the produce and other nutritive necessities of the diet grains, hence witnessed an upsurge in deliberation on whether to opt biological agriculture or conservative agriculture or accept GM crops for advanced produce. Man'svigour and ecological security are the key anxieties of the GM expertise, and in the nonattendance of guarantee by the exponents, countless have considered biological agriculture greater for its advanced harvests as equated to conservative agriculture. Another challenging matter with unswerving harvest yields — monocultures. Monocultures eventually diminish the earth and furnish the prerequisite for extra enrichers and substances to steady harvests. Apparently, this study urges farmers to use deep-rooted farming procedures – like harvest variation and permaculture that nurtured a restored and more maintainable atmosphere for harvests, there would be scarce requirement for all these substances.

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**REVIVEW ARTICLE**

# Role of Schiff base Metal Complexes in Dyes and Paints : A Review

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**ABSTRACT**

Schiff bases are versatile ligands, synthesized by the condensation of an amino compound with a carbonyl compound. Schiff bases and their metal complexes are an interesting area of research that provide valuable information about newly discovered compounds. These compounds and their metal complexes have been extensively studied for their various potential applications in a variety of sectors. This review focuses on Schiff base metal complexes used in dyes and paints.

**Keywords:** versatile ligands, metal, Paints, metal, dyes.

## INTRODUCTION

Schiff bases are the compounds that contain an azomethine group (-CH=N-). They were first discovered in 1964 by Hugo Schiff and are named after his name. These compounds are formed when a primary amine reacts with a carbonyl compound (aldehyde or ketone) Fig.1 showing g eneral methodforpreparation of Schiff base by condensationbetween carbonyl compounds and primary amines.Because of the presence of effective conjugation, aromatic aldehyde Schiff bases are more stable, but aliphatic aldehydes are comparatively unstable and polymerize easily [1]. Schiff base compounds are used in a variety of biological and pharmacological applications. They are also used as fundamental units in several dyes. Transition metal complexes of Schiff bases have been synthesised and studied extensively over the last several decades because they behave as good chelating ligands in the field of coordination chemistry. Chelating ligands comprising N, S, and O donor atoms offer a wide spectrum of biological functions, which may be enhanced by the presence of metal ions attached to them. Because chromium metal

complexes are coloured, they are used in pigments. Furthermore, the colour of emeralds and rubies is caused by traces of chromium ions.Compounds of manganese and nickel are widely used in analytical, metallurgical processes, paint and pigment industriesand ceramics.

The Schiff bases involved in complexation are introduced to demonstrate their use in dyes and pigments. Metal complexes are compared with a newly synthesised Schiff base derived from salicylaldehyde and its derivatives, as well as 2-aminophenol or 2-aminobenzyl alcohol. Various physiochemical techniques were utilised to identify and analyse the characteristics of such compounds [2].The most important characteristic that account for the colour and hard/soft properties of Schiff base reaction sites [2,3,4]is the planar chemical conformation. Several Schiff base transition metal complexes have been synthesised and investigated, such as Al(III), Fe(III), Co(II), Ni(II), and Cu(II). These metal ions, as well as a number of others, have been employed as mordants in dyeing methods. In coordination applications, the principle of complexation and mordent has the same idea. To explain the sites of coordination, the reaction of salicylaldehyde with anthranilic acid or o-aminophenol has been prepared and examined. The reaction represents the interaction of the hydroxyl, amino, and azomethine groups [2,5,6,7,8].

Despite their overall structural similarity, the sort of engagement of the hydroxyl groups and the metal ions in bonding promotes the staining or dyeing activity. The aspect of coordination varifies the sites, components, and groups that can be worked in bonding. The hydroxyl groups, rather than the azomethine groups, are the optimum sites of coordination in this case, as long as these groups are in appropriate positions. Because of the differences in the position and characteristics of the hydroxyl groups, certain Schiff bases may be exceptionally effective agents for the tasks assigned [2].A dye is a coloured substance that chemically bonded to the substrate it is applied on. Dyes are distinguished from pigments by the fact that they do not chemically attach to the substrate they colour. Dye is usually applied in an aqueous solution, and it may be necessary to add a mordant to increase the dye's fastness on the fibre[9].Dye and pigment are both coloured because they absorb only certain wavelengths of visible light. Dyes are generally water soluble, whereas pigments are not. The ability of a dye to impart colours to fibres is its most essential feature. Different types of dyes have been produced for various fibres based on their nature. Different fibres are dyed with different dye classes.

Generally, one ormore class of dyesare used to dye specificfibre. Thefibres are broadly divided into two classes:
(1) Naturalfibres
(2) Syntheticfibres.
Cotton, wool, and silk are examples of natural fibres, whereas synthetic fibres include nylon, cellulose acetate, cellulose triacetate, polyester, polyacrylonitrile, and others. Natural fibres have a strong affinity for water and are hydrophilic in nature. They absorb water and swell, making dyeing with water soluble pigments easier. Reactive dyes, direct dyes, acid dyes, vat dyes, and other dyes are available for dyeing natural fibres. These colours are water soluble and preferentially bind to hydrophilic fibres. Synthetic fibres are hydrophobic in nature, with little or no affinity for water. Synthetic fibres with reactive sites, such as nylon and acrylic fibres, can be dyed with water soluble dyestuffs such as acid dyes and cationic dyes, respectively. On the other hand, the most prevalent synthetic fibres, polyester and cellulose acetate, contributed to the formation of another type of dye known as dispersion dyes.

**Classification of Dyes**
Dyes can be classified in many ways such as according to their chemical structure, the chromophoricsystem, chemical properties, origin and applications. Some of the important classification are described below:

**Classification on the basis of origin:**
**Natural dyes**
The majority of natural dyes come from plants, such as roots, berries, bark, leaves, wood, fungi, and lichens etc.**[10].**
**Synthetic dyes**
The majority of dyes are synthetic in origin, that is, they are man-made and derived mostly from petrochemicals, and they have a wide range of uses. Mauve, the first synthetic dye, was discovered by chance by William Henry Perkin in

1856 **[11,12].** The discovery of Mauveine**Fig. 2(a)** caused an upsurge in synthetic dyes, broadly in organic chemistry. Fuchsine2(b)**,** SafranineFig. 2(c)**,** and Induline were among the other aniline dyes that followed. Since then, dozens of synthetic dyes have been developed **[13,14,15].**

**Chemistry of dyes**

The colour of a dye is determined by its capacity to absorb light in the visible portion of the electromagnetic spectrum (380-750 nm). An early hypothesis known as the Witt theory stated that a colourful dye has two components: a chromophore that gives colour by absorbing light in the visible area (nitro, azo, and quinoid groups are examples) and an auxochrome that serves to intensify the colour. This concept has been replaced by modern electronic structure theory, which states that the colour of dyes is caused by visible light excitation of valence $\pi$-electrons **[**16].

Dyes possess colour because of the following reasons:

1) They absorb light in the visible region of the electromagnetic spectrum in the wavelength ranging from 400nm to 700 nm.
2) They contain at least one chromophoricor colour-bearing group.
3) They have a conjugated system, i.e. the presence of alternate double and single bonds.
4)Theypossess resonance in electronic system which providestabilization to the organic compounds **[17]**.

In the absence of any of the above mentioned distinctive features in the dye's molecular structure, the colour is lost. Chemists have long been interested in finding the connection between a dye's colour and its molecular structure. Furthermore, study of colour and structural relationships has always been crucial in the production of novel dyes, arguably the most significant early contribution to the science of colour characterized as the chromophore and the auxochromes**[**2,18].

The theory based on the chromophore principle is frequently an electron-drawing group and auxochromes are electron-releasing groups that are connected to one another via a conjugated structure. The notion of the donor-acceptor chromogen was therefore formed. Furthermore, it was discovered that increasing the electron-withdrawing power of the chromophore, increasing the electron-releasing power of the auxochromes, and extending the length of the conjugation could result in a bathochromic-shift of the colour, i.e. the absorption band shifting to a longer wavelength. Although it lacks formal theoretical support, the chromophore and auxochrome hypothesis is presented as a straightforward technique for understanding the genesis of colour in dye molecules. The carbonyl (C=O), diazo (-N=N-), azomethine (-CH=N), and nitro (NO2) groups are the most significant chromophores, according to this definition. Other groups, such as hydroxyl (OH) and amino (NR2) groups, intensify the colour and shift absorption to longer wavelengths of light. Most chemical families of dye, including azo, carbonyl, azomethine, and nitro dyes, may be applied to the approach **[2,19].**

**Classification on the basis of chemical properties**:

**Acid dyes**

Acid dyes are anionic dyes that are water-soluble and are applied to fabrics such as silk, wool, nylon, and modified acrylic fibres using neutral to acid dye baths. Salt formation between anionic groups in the dyes and cationic groups in the fibre is thought to be responsible for at least some of the attachment to the fibre. Acid dyes have no effect on cellulosic fibres. The majority of synthetic food colours belong into this group. Acid dyes include Alizarine Pure Blue B, Acid Red 88, and others.

Acid dyes are a large family of dyes that are often formed of sodium salts of colour acids that contain sulphonic acid or phenolic groups. An acid dye's colour is found in its negative ion. These colours are almost often separated as sodium salts. These colours are almost often separated as sodium salts. It is difficult to separate the free dye. Because of their hydroscopic nature, they are difficult to pack and store. Acid dyes are anionic dyes that are water-soluble and are applied to fabrics such as silk, wool, nylon, and modified acrylic fibres using neutral to acid dye baths. The wool and silk textiles are coloured with anionic dyestuffs in an acidic medium. Under these circumstances, the dissociation of the carboxyl group of wool or nylon is limited, and amino groups are transformed to ammonium ion by accepting proton. As a result, the amino groups exist as reactive ammonium ions. In water, the dyestuff dissociates, and its

anion is bonded to silk or wool. Salt formation between anionic groups in the dyes and cationic groups in the fibre is thought to have a role in the dyes' attachment to the fibre.Examples of acid dye are Alizarin(3a),picric acid (3b) , Acid red 88(3c) , Pure Blue B, naphthol yellow, etc.(Fig 3).

**Basic dyes**

Basic dyes are water-soluble cationic dyes that are mostly used on acrylic fibres but also on wool and silk. Acetic acid is typically added to the dye bath to aid in dye absorption onto the fibre. Basic dyes are also employed in paper colouring. Methylene blue 4(a), Crystal violet 4(b), Rhodamine B 4(c), Basic violet 2are some examples ofbasic dyes (Fig 4).

**Direct (Substantive) dyes**

Direct or substantive dyeing is often carried out in a neutral or slightly alkaline dye bath at or near boiling point with the addition of sodium chloride (NaCl), sodium sulphate (Na2SO4), or sodium carbonate (Na2CO3). Cotton, paper, leather, wool, silk, and nylon are all dyed with direct dyes. They are also used to detect pH and as biological stains. Congo red (Fig. 5) ,Indigo, Lichens, and other natural dyes are examples of direct dyes. Direct, azoic, vat, sulphur, and reactive dyes are designed for cellulose polymers. Direct dyes were the first colourants to have affinity for cotton in the absence of a binding ingredient known as a mordant. Many of these dyes have low wet fastness because they are water-soluble. The two primary features of direct dyesare:(1) They are linear molecules, and (2)they can get close to the cellulose chain to enhance the impact of intermolecular interactions like H-bonding.

**Mordant dyes**

Mordant dyes require a mordant, which enhances the dye's resistance to water, light, and sweat. The mordant used is very important since various mordants can significantly alter the final colour. Because the majority of natural dyes are mordant dyes, there is a substantial literature base describing dyeing techniques. The most significant mordant dyes for wool are synthetic mordant dyes, or chrome dyes; these constitute around 30% of wool dyes and are notably effective for black and navy shades. As an after-treatment, potassium dichromate is used as a mordant. It is crucial to highlight that certain mordants, particularly those containing heavy metals, can be dangerous to health and must be used with extreme caution. Examples (Fig 6) of mordant dyes are chrome alum 6(a), alum 6(b), tannic acid, and salts of aluminium, chromium, copper, tin etc.

**Vat dyes**

Vat dyes are basically insoluble in water and hence incapable of directly colouringfibres. However, reduction in alkaline liquor results in the dye's water-soluble alkali metal salt. This form is generally colourless, so in that case it is known as a Leuco dye, and has a strong affinity for textile fibres. Following oxidation, the original insoluble dye is reformulated. Indigo, the original vat dye, is responsible for the colour of denim. Vat dyes get their name from the vatting procedure associated with their application. The structures of vat dyes are well known. They are transformed to a water-soluble form with cellulose affinity and then returned to their water-insoluble form, resulting in high wet-term stability.Indigo, a well-known natural dye, is included in the family of vat dyes. While indigo remains the most significant colourant for denim fabric, its very tiny size makes this vat dye particularly sensitive to removal during the laundry process, giving jeans a faded appearance even after only one wash. This emphasises the need of creating colourants for cellulosic substrates that have the features required to remain inside the polymer matrix when water swells the substrate. Indigo Fig. 7(a), Indanthrene 7(b), Hydro bluvat, are the examples of vat dyes

**Sulphur dyes**

Sulfur dyes are low-cost dyes used to produce dark hues in cotton. Dyeing is accomplished by immersing the cloth in a solution of an organic chemical and sulphide or polysulfide, generally a nitrophenol derivative. When the organic compound combines with the sulphide source, it produces dark colours that attach to the cloth. Sulfur Black 1, the most used dye by volume, lacks a well-defined chemical structure. Sulfur dyes get their name from the fact that sulphur is used in their manufacture. Sulfur dye structures are poorly characterised because their polymeric nature renders them unsuitable for traditional structure characterization techniques. Sulfur dyes are transformed to a water-

soluble form with cellulose affinity and then changed back to their water-insoluble form, providing high wet persistence. Sulfur dyes include (Fig. 8) Sulfur black 1,8(a), Indophenol8(b) etc.

**Reactive dyes**

Reactive dyes have a chromophore attached to a substituent capable of interacting directly with the fibre substrate. Because of the covalent bonds that bind reactive dye to natural fibres, they are among the most lasting of dyes."Cold" reactive dyes, such as Procion MX, Cibacron F, and Drimarene K, are very easy to use because the dye can be applied at room temperature. Reactive dyes are by far the greatest option for dying cotton and other cellulose fibres at home or in the studio. Reactive dyes are the class of suitable colourant for cellulosic fibres. Their name is derived from the fact that they undergo the chemical reaction with cellulose to form a covalent bond. Reactive dyes made it possible to get previously unattainable bright wet-fast shades on cellulosic fibres. Reactive dyes (Fig. 9) include reactive red 1,9(a), reactive blue 19,9(b),reactive red 1809(c),reactive blue 4, reactive orange 5etc.

**Dispersedyes**

Disperse dyes are water-insoluble and were first developed for the dyeing of cellulose acetate. The dyes are finely powdered and marketed as a paste in the presence of a dispersing agent, or spray-dried and sold as a powder. Their primary application is in the dyeing of polyester, although they may also be used to colour nylon, cellulose triacetate, and acrylic fibres. A dyeing temperature of 130 °C (266 °F) is necessary in some cases, and a pressuriseddyebath is employed. The very fine particle size provides a large surface area, which helps in dissolution to allow its absorption by the fiber. The dyeing rate can be greatly influenced by the dispersing agent employed during the grinding process. These dyes are applied to hydrophobic fibres, resulting in an aqueous dispersion. Disperse dyes detach from the reaction mixture as large particles. They are unsuitable for dyeing due to their huge particle size. They provide poor results in this form, thus to obtain good dyeing qualities, the dye must be employed in the form of fine powder and homogeneous, stable suspension. As a result, these colours are finely powdered in the presence of a dispersant before being offered as a paste or powder. The very tiny particle size provides a large surface area, which helps dissolving and allows fibre absorption. Disperse dyes are most commonly used to colour polyester, although they may also be used to colour nylon, cellulose triacetate, and acrylic fibres. Disperse dyes (Fig. 10) include Disperse blue 1,10(a),Disperse blue 3 10(b),Disperse orange 11, 10(c),Disperse orange 25etc.

**Azo dyes**

Azo dyes are those that include one or more azo groups. This is the largest and most important cassl of synthetic colouring substance. These dyes are available in nearly all shades and have a wide variety of uses. They may be used on both natural and synthetic fabrics. Furthermore, many dyes in this class are applied on paper, rubber, leather, and a variety of other materials. Azo pigments and the lakes generated by these azo dyes are widely used in the paint, varnish, lacquer, textile, cosmetic, pharmaceutical, and food sectors. Different metal complexes play an important role in the field of azo compounds. Commercially, copper, chromium, and cobalt complexes of azo dyestuffs bearing metallizable substituents in at least one ortho position relative to the azo group (Fig. 11) or azo dyes produced from metallizable coupling components such as salicylic acid are employed. Many papers have been published on the importance and significance of these metal complex dyestuffs [20,21,22]. Copper and chromium metal complexes are widely used in cotton industries in combination with azo dyes. The azo chrome colours[23]are extremely important in the dyeing of wool. Mordant azo dyes are specifically useful for deep blue, violet, black, brown, and Bordeaux shades.

With chromium as a mordant shade, high-quality, all-around fastness qualities are achieved. Premetallized dye 1:1 chromium azo dyestuffs are being introduced (Fig.12). In 1919, the fast dyes Neolan by Ciba and Palatine by BASF get successed in improving the dyeing procedures and providing improved results in their industrial applications. However, these procedures had a significant disadvantage in that they required a strong acidic dye-bath, which destroyed the wool. Later, German dyestuffs manufacturers overcame this disadvantage to some extent in the 1930s

by using 2:1 chromium and cobalt complexes that required a mildly acidic dye-bath that did not harm the softness of wool [24].

Furthermore, they were suitable for dyeing nylon with a relatively neutral dye-bath. However, the primary challenge was their low solubility, which may be solved by using the Irgalan line of dyestuffs, which are 2:1 chromium and cobalt complex dyestuffs containing methyl sulphonyl, which increases their solubility. Later in 1949, a variety of neutral dyestuffs containing chromium and cobalt dyestuffs with non-ionic solubilizing groups sulphonamide were developed. Likewise, several asymmetric 2:1 chromium complexes comprising two different metallizable azo-dyestuffs with improved fastness and a wide variety of hues were developed. Initially, such dyestuffs were confined to wool and nylon, but with the inclusion of appropriate fibre reactive groups, paint superior fastness to light and wet treatments were accomplished.The azo group has weak donor properties and has not been observed to co-ordinate with a metal atom to form a stable complex unless the metal atom can be held within the dye molecule via chelation with the help of a complex-forming group such as -OH or -$NH_2$ in the ortho position to the azo group [25].

Chromium azomethine complexes [26,27], cobalt complex Schiff base [28], and un-symmetrical complex 1:2 chromium [29] dyes provide fast colours to leathers, food packaging, and wools etc. Azo groups with metal complexes [30] are used to dye cellulose ployester fabrics. To mass dye polyfibers, certain metal complexes [31] are utilised. The cobalt complex of a Schiff base (salicylaldehyde with diamine) has exceptional light resistance and storage properties, and it does not deteriorate even in acidic gases ($CO_2$). A novel tetra dentate Schiff base is used as a chromogenic reagent to determine Ni in natural food samples [32].

## CONCLUSION

In conclusion, Schiff base metal complexes play a crucial role in the advancement of modern dyes and paints. Their vibrant colors, stability, and functional properties make them indispensable in a wide range of applications. The ability to synthesize and customize these complexes further enhances their value, providing industries with the tools to innovate and meet specific needs. As the demand for durable, versatile, and environmentally friendly dyes and paints continues to grow, Schiff base metal complexes will undoubtedly remain at the forefront of this dynamic field.

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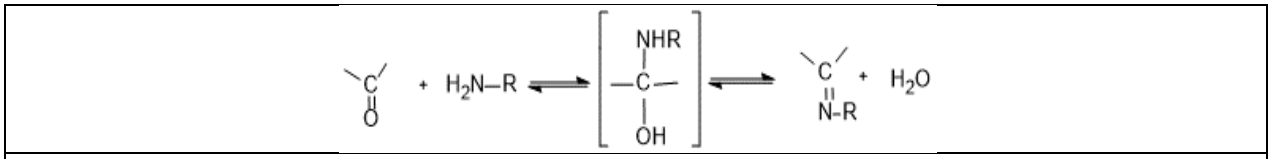



**Fig.1. Preparation of Schiff base**

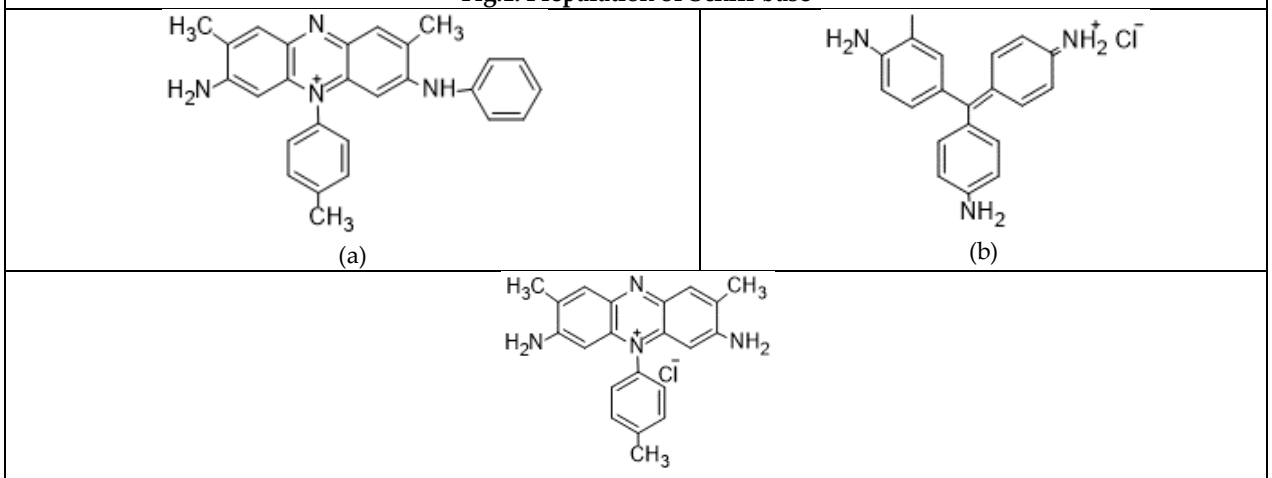
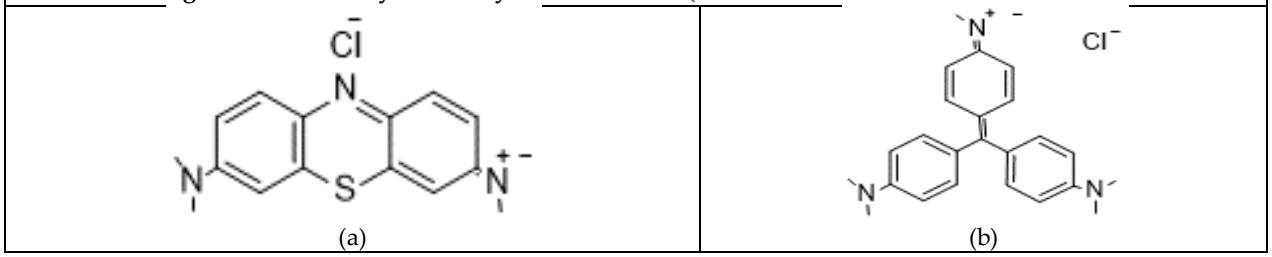
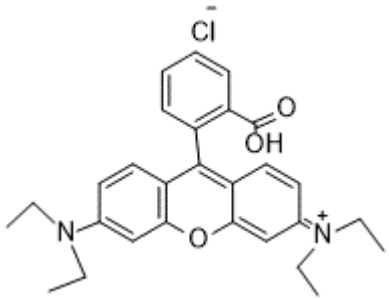



**Fig.2. Structure of synthetic dyes (a) Mauveine (b) Fuchsine (c) Safranine**






(c)

**Fig. 4 Structure of basic dye(a) Methylene blue (b)Crystal voiltet (c)Rhodamine B**

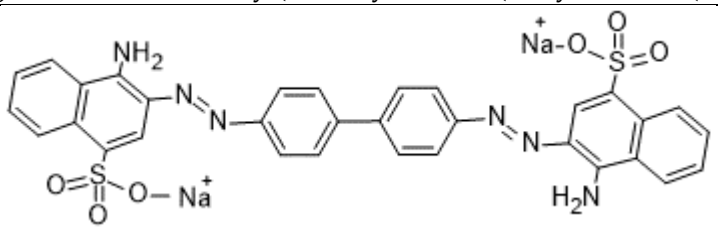
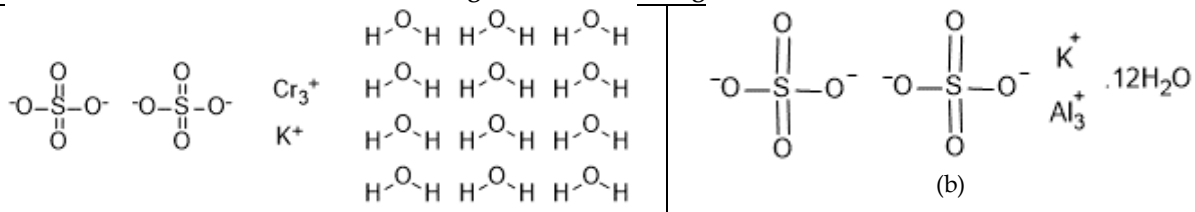
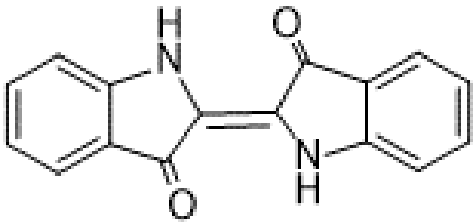



**Fig. 5 Structure of Congo red**

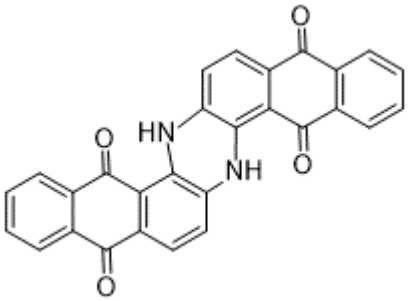



(a) (b)

**Fig. 6. Structureof mordant dyes (a) Chrome alum (b) salts of aluminium**

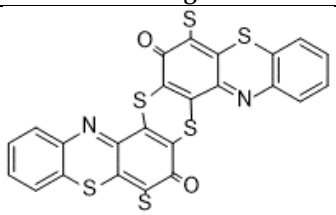


**(a)**

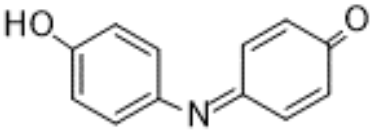



**(b)**

**Fig. 7 Structure of vat dyes(a) Indigo (b)Indanthrene**

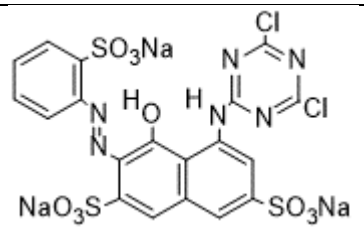


(a)

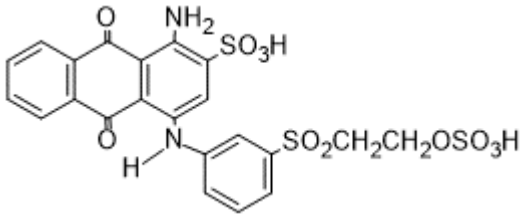



(b)

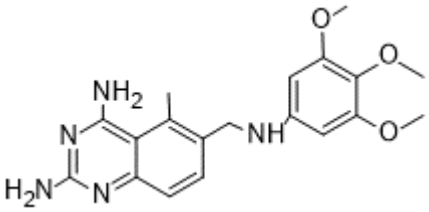
**Fig. 8 Structure of Sulphur dyes(a)Sulfur black 1 (b) Indophenol**



**(a)**

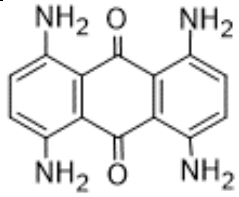


**(b)**

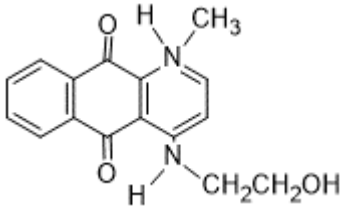


**(c)**

**Fig. 9. Structure of reactive dyes (a) reactive red 1 (b) reactive red 180 (c) reactive blue 4**



**(a)**



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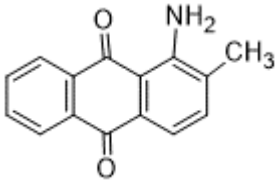


**(c)**

**Fig. 10 structure of (a) Disperse blue 1 (b) Disperse blue 3 (c) Disperse orange 25**

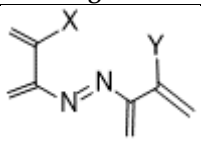
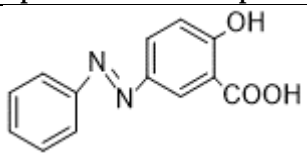
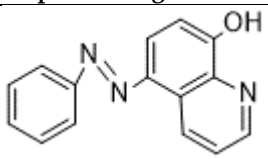


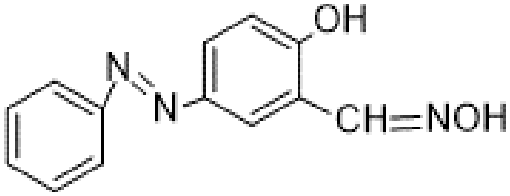
**Fig. 11 Where, X= -OH, -$NH_2$, -OH, -OH, -OH, -OH and Y= -H, -H, -OH, -COOH, -$NH_2$, -$CH_2COOH$ respectively**



**(a)Arylazosalicylic acid**



**(b)Arylazo-8-hydroxyquinoline**



**(c) Arylazosalicyladoxime**

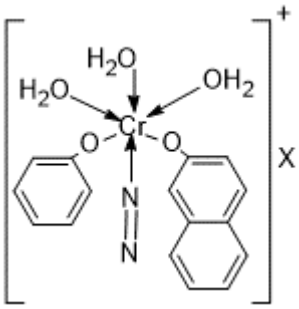


**Fig. 12 Structure of chromium azo dye**

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**REVIEW ARTICLE**

# A Comprehensive Overview of the World Health Organization

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**ABSTRACT**

The World Health Organization [WHO], a public health organization of the United Nations[UN], was founded in Geneva in 1948. Its goal is to help all individuals reach "the highest possible level of health". It sets worldwide sanitary standards and quarantine guidelines, serving as a repository for information on the most recent advancements in illness and medical treatment. It supports efforts to manage endemic and epidemic diseases, such as immunization programmes and aiding in the provision of clean water supplies. Finally, it also promotes the enhancement of public health initiatives within the member countries. Up until this point, its most significant accomplishment has been the global eradication of smallpox. The World Health Assembly [WHA], which represents the 192 WHO member nations, is in charge of the organization. WHO member state delegates make up the Health Assembly. This body approves the WHO's budget and programme and makes major policy decisions.

**Keywords:** WHO; Objectives of WHO; Constitution of WHO; Functions of WHO

## INTRODUCTION

WHO, a UN specialised organization formed in 1948, is dedicated to encouraging global collaboration to enhance public health. With particular responsibilities transferred from the League of Nations Health Organization [1923] and the International Office of Public Health in Paris [1907], the WHO assumed a broad mandate as stated in its constitution: promote the best health for all people. The WHO defines health positively as a condition characterized by total physical, mental, and social well-being, rather than simply the absence of disease or infirmity. WHO observe world Health Day on April 7, 1948, the anniversary of the organization's founding. The majority of the agency's

funding comes from yearly payments given by member nations according to their respective means of subsistence. Furthermore, WHO received significant funding from the UN's enlarged technical assistance programme after 1951[1,2]. The 192 member states of the WHO comprise the World Health Assembly [WHA], which exercises governance over the WHO. The executive council of the WHO is composed of 34 health specialists and is elected and advised by the organization [3,4]. It appoints the chief executive, who is presently Dr.Tedros Adhanom Ghebreyesus of Ethiopia,, establishes objectives while also granting financial and operational permissions [5].The WHO receives the majority of its funding from assessed and voluntary contributions from member states; private donors contribute the remainder. The fiscal year's authorized budget 2020–2021 exceeds $7.2 billion, whereas the budget for the fiscal year 2022–2023 surpasses $6.2 billion [6]. In order to control epidemic and endemic disease, the WHO funds national immunization campaigns, antibiotic and insecticide training, improved lab and clinic facilities for early diagnosis and prevention sanitation systems and rural health education. These programs achieved some success in the battle against AIDS, TB, malaria, and other ailments [7]. The WHO regularly conducts field surveys, sends out teams of experts from around the world to conduct demonstration projects and fieldwork, support for the creation of national training facilities for nurses and doctors, as well as aid with the development of neighbourhood health centres. Additionally, it promotes the growth and improvement of public health administrations across its member countries. WHO is capable of furnishing fellowship funds to medical professionals, public health administrators, sanitary inspectors, nurses, laboratory technicians and researchers via a variety of education assistance programs [8].

**Aim and Objectives**

The WHO goal is for all peoples to achieve the best possible state of health [Figure 1]. The goals of WHO's leadership objectives were:

- Promoting nations' efforts to get closer to achieving universal health care
- Assisting nations in demonstrating their ability to follow international health regulations
- Improving accessibility to necessary and superior medical supplies
- Examining how social, economic, and environmental variables affect public health
- Bringing non-communicable diseases under coordination [9].

**Constitution of WHO**

The states parties to this constitution declare that the following ideas are essential to everyone's pleasure, peaceful coexistence, and security in line with the United Nations Charter. Everyone has the right to good health, regardless of social, political, religious, or economic status. Achieving peace and security depends on ensuring the health of all peoples, which calls for the best possible cooperation between individuals and countries. Every state that succeeds in promoting and protecting health benefits everyone. Unequal progress in promoting health and controlling illness, particularly communicable diseases, throughout nations is a common threat. Children's health depends on peaceful living in a changing environment [10].

**Functions of WHO**

In order to achieve the objectives, the functions of the WHO shall be

- To serve as the international health work's guiding and coordinating authority.
- To support governments in improving health care upon request
- To provide governments with the required help and suitable technical support at their request.
- To promote and further efforts to eliminate endemic, epidemic, and other illnesses.
- To encourage collaboration between professional and scientific communities that progress health.
- Supporting and carrying out health-related research.
- To encourage higher training and teaching standards in the medical, health, and allied fields.
- To create and update worldwide nomenclatures for illnesses, causes of mortality, and public health procedures as needed.
- Standardising diagnostic processes where required.
- To create, implement, and advance international standards for goods that are related to food, biological, pharmaceutical, and other industries.

- In general, to take all required steps to achieve the organization's goal [11,12].

**Governance**

The assembly is the WHO's principal decision-making body. May is the traditional meeting month for representatives from all 192-member nations in Geneva. Its primary duty is to draft the organization's policies. The planned program budget is approved or rejected by the Health Assembly, which also selects the Director-General. The organization's financial policies are under the direct supervision of the Director-General. It also takes into account the findings from the Executive Board, which it advises on issues requiring additional research, analysis, evaluation, or communication. There are 32 members of the executive board that are technically competent in the health profession. Terms of office for members are three years. The primary duties of the board are to advise the health assembly, implement its policies and decisions, and generally facilitate its operation. Approximately 3500 health and other specialists, as well as support personnel, work for the WHO on fixed-term contracts at the organization's headquarters, six regional offices, and in other countries [11,13].

**Director Generals of WHO**

World Health Assembly [WHA]appoints organization's director general. Assembly nominates five-year directors general in May in typical manner. Canadian doctor Brock Chisholm was the organization's first director general from 1948 until 1953. The following directors general of the WHO are Gro Harlem Brundtland [1998–2003], Lee Jong-Wook [2003–2006] and Margaret Chan [2007–17] [14]. Dr.Tedros Adhanom Ghebreyesus, an Ethiopian professional in public health, was appointed director general of the WHO in 2017. He officially began his second term on August 16, 2022, after being re-elected. A director general may be nominated again just once [5].

**Health Policy**

The WHO endeavours to address health disparities by implementing policies and initiatives that prioritize equity, gender responsiveness, and human rights. Additionally, it focuses on fostering a healthier environment, emphasizing primary prevention, and influencing cross-sectoral policies to address underlying causes of environmental health risks. Furthermore, the organization provides evidence-based standards, guidelines, and tools to aid member states in formulating health policies, along with publishing comprehensive reference classifications and ensuring the implementation of the International Health Regulations [15,16].

**Awareness and Action Regarding Public Health**

The WHO defines health education as the process of allowing individuals to raise their level of healthy self-management. It emphasizes social and environmental issues above personal actions. Every year, the organization celebrates World Health Day and conducts health promotion events [Figures 2]. Annually, April 7$^{th}$ marks World Health Day in honor of the organization's founding anniversary. Other internationally recognized public health programs commemorated by the WHO include World Tuberculosis Day, Immunization Week, No Tobacco Day, AIDS Day, and Hepatitis Day. The WHO awards medals and prizes for outstanding contributions to public health. The winners are chosen by the WHO Executive Board and announced during the WHA [17].

**Financing and Partnerships**

Foreign donors and member nations provide financial support to the WHO. The leading donors in 2020–21 included Germany, Japan, China, United States, the Bill & Melinda Gates Foundation, the GAVI alliance, the World Bank, and Rotary International. Advisory Committee on Sustainable Finance was established by the WHO Executive Board in 2021 with the goal of reconsidering WHO's financial approach and submitting suggestions. The WHA of 2022 endorsed its proposals, the most important of which was to increase mandatory member dues to 50% of WHO's basic budget for 2022–2023 by the end of the 2020s [6,18].

**Regional Offices**

The WHO is a specialized UN agency in charge of global public health. Geneva is the headquarters of 150 field offices and 6 regional offices worldwide.

Operating areas and regional offices of WHO:

- Africa; Headquartered in Brazzaville, Republic of the Congo
- Western Pacific; Headquarteredin Manila, Philippines
- South East Asia; Headquarteredin New Delhi, India
- Eastern Mediterranean; Headquarteredin Cairo, Egypt
- Americas; Headquarteredin Washington, D.C., US
- Europe; Head quartered in Copenhagen, Denmark [19,20].

## CONCLUSION

The WHO's stated mission in its constitution is to ensure that all peoples achieve the utmost level of health possible. Ensuring universal access to the advantages of medical, psychological, and related knowledge is critical for achieving optimal health. Active public participation and well-informed opinion are crucial factors in advancing the collective health of the populace. WHO promotes and aids the vulnerable while maintaining global health and safety. The organization promotes health research and policy, sets worldwide health standards, and gathers global health statistics. The organization also helps countries technically. The official World Health Report evaluates global health concerns. The WHO has helped develop an Ebola vaccine, practically eradicate polio, and eliminate smallpox. Non-communicable diseases, including cancer and heart disease; nutrition, health, and food security; substance abuse; and communicable diseases [Ebola, HIV/AIDS, tuberculosis and malaria] are current WHO priorities. The organization supports the health care universalization, active observation of public health risks, coordination of reactions to health crises, and the overall promotion of health and well-being. Thus, in order to promote health, assure global security, and aid the vulnerable, WHO operates on a global scale.

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**RESEARCH ARTICLE**

# Wearable Devices and Digital Health Platforms: Forensic Analysis and Data Security Challenges

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**ABSTRACT**

Smart watches are wearable gadgets that combine the functionalities of a standard watch with those of a smart phone. They usually have a touch screen display, wireless connectivity, and the ability to run apps, track fitness data, and receive notifications from an associated smart phone. Smart watches can perform a variety of duties, depending on the type and software loaded. This article concentrated on health indicators such as heart rate monitoring, breathing activity, sleep quality, and physical activity. This research article seeks to investigate the many factors linked to the usage of smart watches in the domain of health. The effectiveness of health parameters indicated in hardware and software is studied to get insights into the accuracy and usefulness of these metrics.

**Keywords:** Wearable Technology, Health Tracking, Fitness Monitoring, Smart Watches, Device Security

## INTRODUCTION

Forensic science is the use of scientific tools and techniques to investigate crimes and other legal situations. Smartwatches, on the other hand, are wearable devices that may monitor physiological and physical data such as

heart rate, activity levels, and sleep patterns. The possible use of smartwatch data as evidence in criminal investigations connects forensic science and smartwatch readings. For example, if a crime occurred at a given time and location, smartwatch data from a suspect or victim could reveal vital evidence about their whereabouts and activities at that time. Similarly, if a suspect claims to have been asleep at the time of the crime, their smartwatch sleep data might potentially corroborate or contradict their alibi.

However, it is crucial to emphasize that the use of wristwatch data as evidence in judicial disputes is still relatively new, and there are various challenges to be addressed, such as data accuracy and privacy concerns. Furthermore, the admissibility of such evidence in court may be contingent on the specific rules and regulations of a certain jurisdiction. Smartwatches have gone a long way since their introduction in the early 2000s. Microsoft released the first smartwatch, the SPOT watch, in 2004. It was a wristwatch that used FM radio signals to display news, sports scores, and other information. The SPOT watch, on the other hand, was not widely adopted due to its limited functionality and high price [1]. Smartwatches are wearable gadgets that have gained popularity in recent years due to their capacity to perform a variety of purposes such as fitness tracking, messaging, and phone calls, among others. A smartwatch often contains a touch screen display, wireless connectivity, and sensors that can track physical activities and health data. With technological advancements, smartwatches are getting more complex, with capabilities such as voice assistants, mobile payments, and standalone cellular access. [2]According to Allied Market Research, the global smartwatch market was valued at $20.64 billion in 2019 and is expected to reach $96.31 billion by 2027, rising at a CAGR of 19.7% from 2020 to 2027 (Allied Market Research, 2020). The increasing acceptance of wearable devices, as well as people's growing awareness of health and fitness, are some of the factors driving the growth of the smartwatch industry [2]. General information about selected smartwatch for this research work is shown in Table No [1]. Its images are shown in Fig no [1].

**Images of smartwatch used for study**

**Health care Sector vis- a vis data breach**

Health care security refers to the policies and procedures in place to safeguard the confidentiality, integrity, and availability of sensitive personal and medical information of patients and health care providers. This involves security against data breaches, cyber-attacks, theft, and unauthorised access to medical records. [9,10]As electronic health records (EHRs) and other digital technologies are used to store and transmit sensitive patient information, cyber security has become increasingly critical in the healthcare industry. The Health Insurance Portability and Accountability Act (HIPAA) and other requirements require health care organisations to protect patient data, and failing to do so can result in costly data breaches and legal action. Cyber security in healthcare include guaranteeing the confidentiality, integrity, and availability of patient data, as well as safeguarding medical devices and systems from external threats [11, 12]

**Legal implications of wearable devices and digital health platforms**

When you buy a wearable device, you input all of your information into it, and whether or not the data saved is secure becomes a concern. Whether such data is under the control of the device's manufacturer or the service provider. These questions are critical in determining the threats to the privacy rights of individuals storing their data in such devices. In the case of ***K.S. Puttaswamy v. Union of India***, the Supreme Court ruled that the right to privacy is a basic right protected by Articles 14, 19, and 21 of the Indian Constitution. [13] Wearable device and social media network users may not consider themselves to have volunteered data, yet their actions of use and engagement result in the collection of large volumes of data about individual lifestyles, choices, and preferences. Medical information is one type of data that has a reasonable expectation of privacy. This demonstrates that the Court believes in securing medical information about an individual and that it is reasonable for an individual to expect its right to privacy to be violated by anyone. The Digital Information Security in Healthcare Act 2018, often known as DISHA, was passed by the Ministry of Family and Health Welfare. This law would address data owners' rights as well as the gathering and processing of health data. It has also formed a panel of digital health authorities comprised of a National Authority and other State bodies. It focuses on data collected consensually at medical facilities, but it also addresses alternative ways in which medical data is generated.[14] The Information Technology Act of 2000 is the major piece of legislation

in India that governs data protection issues. It is the first legislation that addressed the challenges and, in terms of privacy, developed a notice and consent paradigm. It also imposed fines for violations of data privacy. The Electronic Health Record Standards 2016 are recommended but not legally binding. Many businesses include these in their client data protection policies. In the absence of any national or international norms specific to wearable technologies, it is suggested that wearable technology and service providers have reasonable security practices and procedures in place to protect sensitive personal data and personal information of the user.[15]

**Forensic significance**

**Forensic significance of healthcare data:** Smart watch security is an important consideration that must be treated seriously to safeguard the user's safety and privacy. Smartwatches, like any other computing device, have security vulnerabilities that must be addressed to preserve the user's safety and privacy. Data Privacy, Authentication, Secure Communication, App Security, Physical Security, and Software Updates are the security components of smartwatches that must be considered.[16,17,18,19]

**Forensic significance of smartwatch:** Due of the abundance of data they can supply, smartwatches can be important instruments in forensic investigations. Smartwatches, in particular, can provide location data, heart rate tracking, and biometric data that can be utilised in investigations. Smartwatch location tracking features can be utilized to reconstruct a suspect's movements and give evidence in a criminal investigation. [20] Smartwatch heart rate monitoring data can be utilised to corroborate an individual's alibi or dispute a suspect's assertions. [21] Finally, biometric data gathered by smartwatches, such as fingerprints or facial recognition, can be useful in forensic investigations. [22] Overall, smartwatches can give valuable forensic evidence in investigations and should be considered while conducting investigations.

## METHODOLOGY

Aims and objectives: To investigate the effectiveness of health measures indicated in hardware such as smartwatches and software.

We determined to work on these hardware wearable devices: Fire Boltt BSW007, Gadgetzone I8 Pro Max, Gizmore GIZFIT 907, Noise Color Fit Pro 2 and Boat Wave Beat, as well as five software applications related to health: Heart Rate Monitor-Pulse App, Step counter-Pedometer, GoogleFit: Activity Tracking, Blood Pressure Measurement App, and Samsung Health. Certain basic data such as Blood Pressure (BP), Heart Rate, Blood Oxygen, and some training parameters such as cycling, skipping, and so on are measured for 15 days utilising the aforementioned hardware wearables. Software health programmes measure heart rate, step count, blood pressure, and calories count for 5 days. A detailed workflow is shown in Fig No.[2]

**Proposed Methodology**

Show the Fig.[2] -Workflow approach of proposed exploratory research

**Experimental Methodology**

**Sample size**–06 persons with one Smart watch from six different companies were selected.

**Age Group** - between the ages of 18 and 25 was chosen where,

Population Inclusion - The general population aged 18 to 25 years.

Population Exclusion - We excluded pregnant ladies, HIV patients, cancer patients, and anyone with any form of condition or sickness.

**Process-** For a period of 15 days (25.02.2023), the health parameters were examined at any time every day.

This investigation concentrated on both hardware and software. In terms of hardware devices, health parameters selected for investigation are classified into two categories: basic parameters and training parameters. Observations are shown in Table No. [2] For basic parameters and Table No.[3] for training parameters. As far as software is concerned, Table No.[4] Displays the Software application reading.

**Hardware Devices**

**Basic Parameters**

1. Blood Pressure(diastolic, systolic mm/hg(millimeter of mercury )), 60/90
2. Heart Rate BPM(Beats per minute) , 60-100 bpm
3. sleep(hours and minutes), 8 hours
4. walking steps(steps), target 7000 steps/day
5. Blood oxygen Level(% percentage ),95-100%
6. Outdoor running, mileage as per Time(Hrs and Min), Pace(min/km), consumption of calorie(Kcal)
7. Female Menstrual Cycle Tracking,
8. Drink water reminder

**Training Parameters**

1. Cycling (bpm and KCAL)
2. Swimming
3. Football(steps, bpm and KCAL)
4. Skipping (bpm and KCAL)
5. Badminton (steps, bpm and KCAL)
6. Basketball (steps, bpm and KCAL)

**Software Application**

1. Heart Rate Monitor-Pulse App
2. Step counter-Pedometer
3. GoogleFit: Activity Tracking
4. Blood Pressure Measurement App
5. Samsung Health

**Basic parameters reading of smartwatch:**

**Show the details in Table.2**

**Training parameters reading of smartwatch:**

**Observation table for software apps** The Table No [5] below compares medical expert readings to one-day smartwatch readings. On 18 April 2023, the readings are recorded.

## CONCLUSION

Finally, this article work provides useful insights into the forensic investigation of smartwatches. The study emphasizes the need of taking into account the health parameters shown in smartwatches and software, as well as the security features they provide. It is critical to do forensic analysis on smartwatches in order to analyses any occurrences that occur while wearing them. This study emphasizes the need of forensic scientists in reviewing available digital data to identify critical evidence in criminal investigations. The project work emphasizes the importance of continuing to engage in forensic science research in order to keep up with the rapid development of wearable technology devices such as smartwatches. This study was conducted on a typical population; we eliminated pregnant women, HIV patients, people with deviant behavior, and anyone with any type of disease. Smartwatches also capture data about the wearer's health and fitness, such as heart rate, sleep habits, and activity levels. This data can be utilized to establish the wearer's physical status at a given time and may be significant in cases involving physical assaults or accidents. Hardware and software conclusion: The outcomes of this study will be based on the effectiveness of health parameters indicated in hardware such as smartwatches and software. Depending on the data, the conclusion could be that these devices and software are helpful in tracking and monitoring health metrics, or that they are not dependable enough to offer accurate readings. The conclusion would almost certainly include recommendations for additional research and development to increase the accuracy and reliability of these health-monitoring systems. According to health experts, software and smart gadgets have a 10-20% error rate. In reality, traditional procedures must be employed to obtain 100% precise health metric data, and

individualized treatment is advised based on a variety of environmental and situation factors. Another issue is that if one is continually reviewing the health findings, they risk making individuals uneasy and putting them in danger.

## DISCUSSION

• Due to time constraints, only 6 smartwatches from diverse manufacturers and 5 software applications were considered for the study.
• According to health experts, software and smart gadgets make 10-20% of errors. In reality, traditional procedures must be employed to obtain 100% precise health metric data, and customized therapy is advised based on a variety of environmental and situation factors. Another issue is that if one is continually reviewing the health findings, they risk making individuals uneasy and putting them in danger.
• We eliminated pregnant women, HIV patients, those with deviant behavior, and people with any form of disease from this study.

**List of Abbreviations**

| | | | |
|---|---|---|---|
| NA | -Not Applicable | Cal | -Calories |
| BP | -Blood Pressure | % | -Percentage |
| BPM | -Beats Per Minute | mmHg | -Millimeter(s) of mercury |
| SBP | -Systolic Blood Pressure | DBP | -Diastolic Blood Pressure |
| & | -and | Yr/Yrs | -Year/Years |

**Competing Interests**
"The authors Ms. Swagata Shashikant Zarkar, Mr. Rahul Kailas Bharati, Dr. Shobha Kamalakar Bawiskar declares that they have no competing interests" We are not receiving or having financial competing and non-financial competing interests
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**Disclaimer:**
Researcher only conducts a pilot study on various available hardware and software tools In this research, researcher never encourages / do not promote / do not advertise any specific applications.

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**Table No.1 General information about smartwatches used for study.**

| Sr.No. | Features of watch | Device 1 Fire Boltt BSW007 [3] | Device 2 Boult Drift Bluettoth Calling [4] | Device 3 Gadgetzone I8 Pro Max [5] | Device 4 Gizmore GIZFIT 907 [6] | Device 5 Noise ColorFit Pro 2 [7] | Device 6 Boat Wave Beat [8] |
|---|---|---|---|---|---|---|---|
| 1 | Price @ 19 April 2023 | 1,599 | 1,799 | 840 | 1,899 | 1,199 | 1,499 |
| 2 | Water resistant | Yes | Yes | Yes | Yes | Yes | Yes |
| 3 | Scratch resistant screen | No | No | No | No | No | No |
| 4 | Crash detection and emergency SOS | No | No | No | No | No | No |
| 5 | Battery life | 5 days | 10 days | 10 days | 12 days | 10 days | 7 days |
| 6 | Advanced sensors | Yes | Yes | Yes | Yes | Yes | Yes |
| 7 | ECG tracker | No | No | No | No | No | No |
| 8 | Design | Full metal body &ultra | Lightweight | Lightweight | Lightweight | Stylish & lightweight | Slim metallic |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | lightweight | | | | | design |
| 9 | Display size | 1.3 inches | 1.69 inches | 44 mm | 14 inches | 1.3 inches | 1.69 inches |
| 10 | Track sleep | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | GPS system | No | No | No | No | No | No |
| 12 | Calling | No | Yes | Yes | No | No | No |
| 13 | Mobile payment | No | No | No | No | No | No |
| 14 | Compatibility OS-iOS, Android | Android &iOS | Android &iOS | Android &iOS | Android &iOS | Android &iOS | Android &iOS |
| 15 | Music | No | Yes | Yes | No | No | Yes |
| 16 | Connectivity | Bluetooth | Bluetooth | Bluetooth | Bluetooth | Bluetooth | Bluetooth |
| 17 | Touchscreen | Yes | Yes | Yes | Yes | Yes | Yes |

**Table No.2 Basic parameters reading of smartwatch.**

| **Sr. no.** | **Basic parameters** | **Measuring units** | **Nor-mal range** | **Date 2023** | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** | **Sample 6** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Device 1 Fire Boltt** | **Device 2 Boult Drift** | **Device 3 I8 Pro Max** | **Device 4 Gizmore** | **Device 5 Noise** | **Device 6 Boat Wave Beat** |
| | | | | | **Age-22yrs** | **Age-18yrs** | **Age-22yrs** | **Age-22yrs** | **Age-22yrs** | **Age-22yrs** |
| | | | | | **Female** | **Male** | **Female** | **Female** | **Female** | **Female** |
| 1 | **Blood Pressure** | Systolic /Diastolic mm/Hg (millimeter of mercury) | 120/80 mm/Hg | 25/2 | 123/77 | 114/71 | 97/77 | NA | NA | NA |
| | | | | 26/2 | 119/79 | 111/74 | 117/67 | NA | NA | NA |
| | | | | 27/2 | 132/73 | 111/74 | 102/72 | NA | NA | NA |
| | | | | 28/2 | 110/68 | 120/72 | 126/76 | NA | NA | NA |
| | | | | 1/3 | 114/71 | 119/77 | 121/66 | NA | NA | NA |
| | | | | 2/3 | 122/76 | 117/73 | 126/71 | NA | NA | NA |
| | | | | 3/3 | 116/72 | 120/72 | 124/79 | NA | NA | NA |
| | | | | 4/3 | 132/73 | 117/74 | 117/67 | NA | NA | NA |
| | | | | 5/3 | 128/70 | 120/78 | 96/66 | NA | NA | NA |
| | | | | 6/3 | 119/79 | 118/73 | 121/66 | NA | NA | NA |
| | | | | 7/3 | 144/82 | 120/71 | 98/68 | NA | NA | NA |
| | | | | 8/3 | 113/70 | 117/71 | 127/82 | NA | NA | NA |
| | | | | 9/3 | 103/64 | 118/72 | 108/68 | NA | NA | NA |
| | | | | 10/3 | 129/76 | 119/70 | 103/78 | NA | NA | NA |
| | | | | 11/3 | 116/72 | 111/71 | 97/77 | NA | NA | NA |
| 2 | **Heart rate** | Bpm (beats per minutes) | 60-100 bpm | 25/2 | 63 | 58 | 95 | 83 | 91 | 85 |
| | | | | 26/2 | 90 | 70 | 87 | 86 | 118 | 79 |
| | | | | 27/2 | 75 | 73 | 95 | 84 | 65 | 101 |
| | | | | 28/2 | 72 | 87 | 88 | 88 | 68 | 91 |
| | | | | 1/3 | 73 | 75 | 94 | 82 | 62 | 92 |
| | | | | 2/3 | 108 | 75 | 71 | 90 | 66 | 81 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 3/3 | 73 | 76 | 94 | 85 | 72 | 81 |
| | | | | 4/3 | 86 | 75 | 71 | 73 | 71 | 86 |
| | | | | 5/3 | 80 | 78 | 89 | 87 | 111 | 82 |
| | | | | 6/3 | 68 | 80 | 89 | 85 | 74 | 82 |
| | | | | 7/3 | 79 | 76 | 97 | 85 | 77 | 74 |
| | | | | 8/3 | 72 | 77 | 80 | 84 | 78 | 65 |
| | | | | 9/3 | 65 | 78 | 73 | 83 | 111 | 84 |
| | | | | 10/3 | 83 | 80 | 76 | 88 | 77 | 95 |
| | | | | 11/3 | 72 | 78 | 88 | 88 | 101 | 75 |
| 3 | **Sleep** | Hours & minutes | 8 hrs. | 25/2 | 5.24 | 7.57 | 4.56 | 6.45 | 6.22 | 5.00 |
| | | | | 26/2 | 8.00 | 7.30 | 7.11 | 4.50 | 4.60 | 8.59 |
| | | | | 27/2 | 6.45 | 8.07 | 5.59 | 8.09 | 4.29 | 7.11 |
| | | | | 28/2 | 6.38 | 6.36 | 4.30 | 6.59 | 7.01 | 6.33 |
| | | | | 1/3 | 7.09 | 8.22 | 5.59 | 7.03 | 6.45 | 7.45 |
| | | | | 2/3 | 8.34 | 5.59 | 8.30 | 4.30 | 7.32 | 7.32 |
| | | | | 3/3 | 8.56 | 11.04 | 6.25 | 5.32 | 5.14 | 5.00 |
| | | | | 4/3 | 7.06 | 6.45 | 7.34 | 5.45 | 5.59 | 3.58 |
| | | | | 5/3 | 5.10 | 6.38 | 7.00 | 8.00 | 4.16 | 7.51 |
| | | | | 6/3 | 5.29 | 10.16 | 7.38 | 5.41 | 5.10 | 9.28 |
| | | | | 7/3 | 8.00 | 4.50 | 3.59 | 7.00 | 3.30 | 5.45 |
| | | | | 8/3 | 6.30 | 7.17 | 7.45 | 4.56 | 4.56 | 7.09 |
| | | | | 9/3 | 7.12 | 7.56 | 8.06 | 6.43 | 7.43 | 6.56 |
| | | | | 10/3 | 4.00 | 9.48 | 6.45 | 6.56 | 6.45 | 6.50 |
| | | | | 11/3 | 7.52 | 6.23 | 6.26 | 5.05 | 8.56 | 6.43 |
| 4 | **Walking steps** | Steps | 7000 steps per day | 25/2 | 6000 | 4500 | 5322 | 3578 | 5000 | 5476 |
| | | | | 26/2 | 4515 | 4521 | 4356 | 6212 | 3500 | 2390 |
| | | | | 27/2 | 5784 | 1698 | 6500 | 4100 | 6078 | 2178 |
| | | | | 28/2 | 2500 | 3421 | 3845 | 1900 | 5040 | 7000 |
| | | | | 1/3 | 6587 | 7054 | 2389 | 7000 | 5006 | 3421 |
| | | | | 2/3 | 7000 | 2856 | 6500 | 3053 | 6990 | 5478 |
| | | | | 3/3 | 1500 | 5743 | 4890 | 4508 | 1500 | 6110 |
| | | | | 4/3 | 2467 | 2000 | 3810 | 6100 | 4675 | 6577 |
| | | | | 5/3 | 1900 | 4106 | 6987 | 3217 | 5087 | 4390 |
| | | | | 6/3 | 1545 | 2080 | 2756 | 2590 | 6832 | 3749 |
| | | | | 7/3 | 3000 | 1700 | 2990 | 2985 | 3452 | 3241 |
| | | | | 8/3 | 7995 | 2167 | 5643 | 7000 | 3323 | 3677 |
| | | | | 9/3 | 5330 | 6432 | 5512 | 3465 | 1990 | 5470 |
| | | | | 10/3 | 7000 | 3505 | 3412 | 4822 | 1450 | 5623 |
| | | | | 11/3 | 2590 | 3080 | 3980 | 6471 | 4532 | 6432 |
| 5 | **Blood oxygen level** | Percentage | 95-100% | 25/2 | 97 | 98 | 95 | 96 | NA | 98 |
| | | | | 26/2 | 96 | 97 | 95 | 98 | NA | 97 |
| | | | | 27/2 | 98 | 97 | 98 | 98 | NA | 99 |
| | | | | 28/2 | 98 | 97 | 97 | 94 | NA | 98 |
| | | | | 1/3 | 97 | 96 | 98 | 90 | NA | 99 |
| | | | | 2/3 | 96 | 96 | 98 | 94 | NA | 97 |
| | | | | 3/3 | 97 | 98 | 97 | 97 | NA | 98 |
| | | | | 4/3 | 98 | 96 | 98 | 98 | NA | 97 |
| | | | | 5/3 | 98 | 98 | 96 | 96 | NA | 98 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 6/3 | 97 | 95 | 96 | 95 | NA | 98 |
| | | | | 7/3 | 98 | 94 | 98 | 98 | NA | 98 |
| | | | | 8/3 | 96 | 95 | 95 | 97 | NA | 94 |
| | | | | 9/3 | 96 | 94 | 97 | 96 | NA | 97 |
| | | | | 10/3 | 98 | 96 | 98 | 90 | NA | 99 |
| | | | | 11/3 | 97 | 95 | 95 | 97 | NA | 97 |
| 6 | **Running (Indoor/outdoor)** | As per time – hours & minutes | - | - | Not used | NA | NA | NA | NA | NA |
| | | Pace – min/km | - | - | Not used | NA | NA | NA | NA | NA |
| | | Consumption of calorie-Kcal | - | - | Not used | NA | NA | NA | NA | NA |
| 7 | **ECG tracker** | | - | - | NA | NA | NA | NA | NA | NA |
| 8 | **Female menstrual cycle tracking** | - | - | - | NA | Not used | NA | NA | Not used | NA |
| 9 | **Stress 0, 6, 12, 24 hrs.** | 33:66:99 (Low: Average: Medium) | - | - | NA | NA | NA | NA | NA | NA |
| 10 | **Mood swing** | - | - | - | NA | NA | NA | NA | NA | NA |
| 11 | **Drink water remainder** | - | As per Condition | - | NA | Not used | NA | Not used | NA | NA |

**Table No. 3 – Training parameters reading of smartwatch**

| **Sr. no.** | **Training parametrs** | **Units of measuring** | **Normal range** | **Date** | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** | **Sample 6** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Device 1 Fire Boltt** | **Device 2 Boult Drift** | **Device 3 I8 pro max** | **Device 4 Gizmore** | **Device 5 Noise** | **Device 6 Boat wave beat** |
| | | | | | **Age-22yrs** | **Age-18yrs** | **Age-22yrs** | **Age-22yrs** | **Age-22yrs** | **Age-22yrs** |
| | | | | | **Female** | **Male** | **Female** | **Female** | | |
| 1 | **Cycling** | Bpm/ KCAL | 60/90 | 25/2 | Not used | NA | Not used | NA | Not used | 79/5 |
| | | | | 26/2 | - | NA | - | NA | - | 110/16 |
| | | | | 27/2 | - | NA | - | NA | - | 73/5 |
| | | | | 28/2 | - | NA | - | NA | - | 78/24 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1/3 | - | NA | - | NA | - | 63/18 |
| | | | | 2/3 | - | NA | - | NA | - | 90/29 |
| | | | | 3/3 | - | NA | - | NA | - | 65/10 |
| | | | | 4/3 | - | NA | - | NA | - | 80/44 |
| | | | | 5/3 | - | NA | - | NA | - | 72/7 |
| | | | | 6/3 | - | NA | - | NA | - | 88/14 |
| | | | | 7/3 | - | NA | - | NA | - | 80/25 |
| | | | | 8/3 | - | NA | - | NA | - | 72/13 |
| | | | | 9/3 | - | NA | - | NA | - | 73/8 |
| | | | | 10/3 | - | NA | - | NA | - | 78/15 |
| | | | | 11/3 | - | NA | - | NA | - | 69/10 |
| 2 | **Swimming** | KCAL | 60/100 bpm | - | Not used | NA | NA | NA | NA | NA |
| 3 | **Football** | Steps, Bpm KCAL | 8 hrs | - | Not used | NA | NA | NA | NA | Not used |
| 4 | **Skipping** | Bpm/ KCAL | - | 25/2 | 80/16 | NA | NA | NA | NA | NA |
| | | | | 26/2 | 95/24 | NA | NA | NA | NA | NA |
| | | | | 27/2 | 75/8 | NA | NA | NA | NA | NA |
| | | | | 28/2 | 87/18 | NA | NA | NA | NA | NA |
| | | | | 1/3 | 90/20 | NA | NA | NA | NA | NA |
| | | | | 2/3 | 86/15 | NA | NA | NA | NA | NA |
| | | | | 3/3 | 75/12 | NA | NA | NA | NA | NA |
| | | | | 4/3 | 75/6 | NA | NA | NA | NA | NA |
| | | | | 5/3 | 89/14 | NA | NA | NA | NA | NA |
| | | | | 6/3 | 72/9 | NA | NA | NA | NA | NA |
| | | | | 7/3 | 90/22 | NA | NA | NA | NA | NA |
| | | | | 8/3 | 79/10 | NA | NA | NA | NA | NA |
| | | | | 9/3 | 108/27 | NA | NA | NA | NA | NA |
| | | | | 10/3 | 74/9 | NA | NA | NA | NA | NA |
| | | | | 11/3 | 85/12 | NA | NA | NA | NA | NA |
| 5 | **Badminton** | Steps, Bpm KCAL | 95-100% | - | Not used | NA | NA | NA | NA | Not used |
| 6 | **Hiking** | - | - | - | NA | NA | NA | NA | NA | NA |
| 7 | **Cricket** | - | - | - | NA | NA | NA | NA | NA | Not used |
| 8 | **Yoga** | - | - | - | NA | NA | NA | NA | NA | Not used |
| 9 | **Rower** | - | - | - | NA | NA | NA | NA | NA | NA |
| 10 | **Elliptical** | - | - | - | NA | NA | NA | NA | NA | NA |
| 11 | **Workout** | - | - | - | Not used | Not used | NA | NA | NA | NA |
| 12 | **Breath training (inhale, exhale)** | - | Minimum, medium, fast | - | NA | NA | NA | Not used | Not used | NA |

**Table No.4 – Software app readings**

| Sr. No. | Name of the app | Reviews | Ratings | Rating Date | Parameter's measure | Readings |
|---|---|---|---|---|---|---|
| 1. | Heart Rate Monitor-Pulse App [23] | 4.7 | 3+ | 20 April 2023 | Check heart rate (BPM) | 7-4-2023 80 bpm |
| | | | | | | 8-4-2023 100 bpm |
| | | | | | | 9-4-2023 93 bpm |
| | | | | | | 11-4-2023 129 bpm |
| | | | | | | 13-4-2023 85 bpm |
| 2. | Step counter-Pedometer [24] | 4.8 | 3+ | 19 April 2023 | Auto tracks your daily steps & calories. | 7-4-2023 4589 steps |
| | | | | | | 8-4-2023 55 steps |
| | | | | | | 9-4-2023 3276 steps |
| | | | | | | 10-4-2023 1590 steps |
| | | | | | | 11-4-2023 4562 steps |
| 3. | GoogleFit: Activiy Tracking [25] | 4.3 | 3+ | 19 April 2023 | Track your physical activity like calories count. | 9-4-2023 1271 cal |
| | | | | | | 10-4-2023 1271 cal |
| | | | | | | 11-4-2023 1271 cal |
| | | | | | | 12-4-2023 1271 cal |
| | | | | | | 13-4-2023 1271 cal |
| 4. | Blood Pressure Measurement App [26] | 4.7 | 3+ | 20 April 2023 | Measures the blood pressure (systolic/diastolic). | 15-4-2023 88/65 |
| | | | | | | 16-4-2023 102/79 |
| | | | | | | 17-4-2023 90/77 |
| | | | | | | 18-4-2023 93/79 |
| | | | | | | 20-4-2023 80/84 |
| 5. | Samsung Health [27] | 4.2 | 3+ | 20 April 2023 | Track your fitness, weight, diet, food and sleep. | 16-4-2023 3463 steps |
| | | | | | | 17-4-2023 4230 steps |
| | | | | | | 18-4-2023 3237 steps |
| | | | | | | 19-4-2023 1340 steps |
| | | | | | | 20-4-2023 49 steps |

**Table No. 5 – Smartwatch readings and medical expert readings**

| Health parameters | Smartwatch readings | | | Medical expert reading | | |
|---|---|---|---|---|---|---|
| | Blood Pressure (SBP/DBP) | Heart rate (BPM) | Blood oxygen (%) | Blood pressure | Heart rate | Blood oxygen |
| Device 1 | 110/82 | 66 | 98 | 110/75 | 75 | 97 |
| Device 2 | 132/73 | 91 | 99 | 120/78 | 79 | 99 |
| Device 3 | 96/66 | 89 | 95 | 110/70 | 80 | 96 |
| Device 4 | NA | 86 | 90 | NA | 79 | 94 |
| Device 5 | NA | 77 | NA | NA | 75 | NA |
| Device 6 | NA | 85 | 99 | NA | 80 | 98 |

Fig[1.1]- Fire Boltt smartwatch

Fig[1.2]- Boult Drift Smartwatch

Fig[1.3]-I8 Pro Max smartwatch

Fig[1.4]- Gizmore smartwatch

Fig[1.5]- Noise smartwatch

Fig[1.6]- Boat Wave Beat smart watch

**Fig. 1 Smartwatch images for research Work**

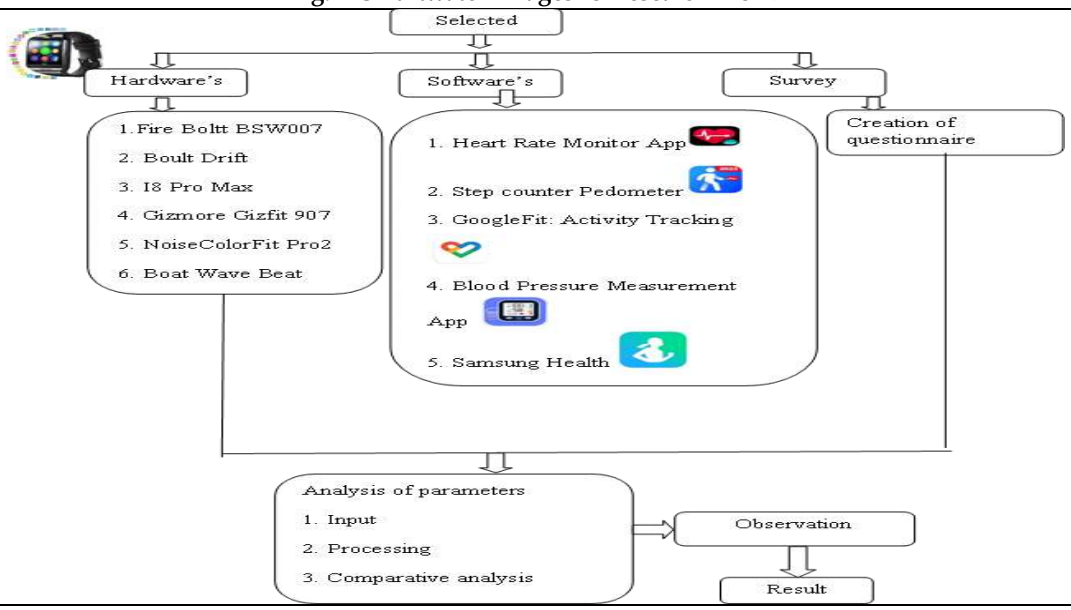


**Fig.2 -Workflow approach of proposed exploratory research**

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**RESEARCH ARTICLE**

# An Effect of Virtual Reality in HRD - IT Sector

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**ABSTRACT**

In today's competitive IT sector, the effectiveness of training and development programs for employees is paramount. This study explores the dimensions in Virtual Reality for effective training and development strategies tailored specifically for IT employees. Through a comprehensive literature review and empirical analysis, this research investigates the effect of training and development that initiatives on employee performance, job satisfaction, and overall organizational success within the IT industries. Moreover, the study identifies key factors influencing the design, implementation, and evaluation of training programs, including technological advancements, regulatory requirements, and changing customer expectations. By synthesizing theoretical insights with practical implications, this research provides valuable insights for IT managers and HR professionals to optimize training and development efforts with VR, enhance employee competencies, and ultimately drive organizational growth in the dynamic IT landscape.

**Keywords**: Effective, Training, Development, Virtual Reality, Performance and Satisfaction

## INTRODUCTION

The Human Resources is the most vital resources for an organization as they are considered essential to accomplish the goals of a business. The growth of IT sector is important for its survival in the today's competitive world, thus the employees of the firm have to enhance their prevailing skills. In today's technological world in order to the improve the skill of an employee in an effective way, the extent of the chosen training programme should reflect on the efficiency of the employee performance. Hence virtual training has its uniqueness and innovativeness within it as the pivotal role played by continuous learning in the IT sector. In an ever-evolving financial landscape, the need for

skilled and adaptable employees is paramount. By addressing the challenges in the sector, such as regulatory compliance and technological advancements, the research endeavors to provide valuable insights for optimizing the professional growth for IT employees by ensuring their sustained success in a dynamic financial environment. The study aims to explore methodologies and tools that optimize Virtual training programs, ensuring alignment with industry trends and regulatory requirements. It will investigate the impact of personalized learning, simulation techniques, and continuous feedback on employee performance.

**Virtual Reality**

It is a technology which has shown prospective transformation in various sectors including Human Resource in recent days with its unique and practical training session. It can be used by the users using electronic gadgets like headset. It is the updated version from the Ivan Sutherland, a computer Scientist's "The Sword Of Damocles" in 1960s. In course of time, it has become as powerful tool by providing immersive learning practice.

**Role of VR In HR Training**

The role of VR in Human Resource Development is border and its role in training and development is to develop a interactive learning practice. Here interactive based training is provided to enhance the communication, leadership, compliances etc.. Leadership can be simulated by strategic thinking and decision making skills with diversification of perspectives and circumstances which will make one to feel the empathy. Apart from this VR helps the on boarding new employees to understand the culture and policies prevailing in the organization. It is cost effective training method for an organization in many aspects. Employee engagement can also be made effective using VR process. In real-time the usage of Virtual Reality in the HR has many benefits along with the cost-cut benefits for the management of an organization.

It is a evolutionary technique used in Training and development within the IT sector to enhance various major domains like Learning practice, realistic skill and to overcome hurdles in the training process. Exhilarating Training Atmosphere: VR training system uses real time scenario and allow the employees to communicate with virtual representatives. It is easier for the employee to understand the difficult model in a effective way than the traditional training method. Real Time Experience: VR enhance the IT professional's problem solving skills by experiencing the real world situation without the risk of reside system. Safe learning environment give them self-assurance and confidence to make mistakes and correct it.

Scalable Training plan: VR provides training program to numerous users at the same time or at the flexible time of the user beside the location barrier and the training programme can be modified according to the learner needs. Assessment and Feedback : VR is real time learning system which can evaluate the performance of an employee in each step and give the feedback to them accordingly based upon their performance in the assessment. Reduce Training Cost: implement of VR technology include huge cost whereas the VR provide training for long run which reduce the cost of training.

**Research Objectives**

Evaluate the existing Virtual training programs within the IT to identify strengths and weaknesses.

Determine the specific skills and knowledge gaps among IT employees that hinder optional performance.

**Research Scope:**

1. The study will focus on a specific region or multiple regions, analyzing the training and development practices within the IT sector.
2. The scope will cover employees at various levels, from entry-level positions to managerial roles, to ensure a comprehensive understanding of training needs.
3. Determine the training needs related to staying abreast of changing regulations and compliance requirements in the financial industry.

## REVIEW OF LITERATURE

A literature review serve as a important component of academic research, providing a widespread overview of existing scholarly literature on a particular area. It act as a major role in identifying gaps, trends, and debates within the field, informing the research questions, methodology, and theoretical framework of a study. (Mantovani, 2004) Considering the ongoing advancement in information technology, the rise of the internet, its widespread use, and the variety of its special services for information distribution and transmission, it is now one of the most widely used information sources. With the rise of e-learning technologies and the internet as a major tool for program delivery, remote learning has become possible in the medical and health care. (Fabrizia Mantovani 1, 2003) the organization of education for health professionals is being significantly impacted by new developments in health care delivery. It is widely acknowledged that medical knowledge doubles every 7-8 years and that new medical methods are developed on a daily basis .There are more and more challenges resources learning resources available thanks to recent development in educational technology. In fact, VR training may help to increase trainees' interest and motivation as well as providing a rich, dynamic and captivating instructional environment that support experiential learning by doing.

## SUGGESTIONS

The awareness about the VR should be provided to the employees so as to improve the efficiency of it. The IT sector can give training programs to their employees to fill the prevailing communication gap within their colleagues. The superior should motivate their employees in their learning. The training provided to the employees can be effective so that they can implement their learning without seeking support from their superior.

## CONCLUSION

Effective training and development programs are essential for the sustained success of ITs in today's competitive environment. Through this study, it is evident that investing in employee . Training not merely improves individual performance but also enhance the overall organizational effectiveness. By aligning training initiatives with strategic goals and identifying the evolving needs of employees, ITs can cultivate a atmosphere of continuous learning and innovation. Additionally, leveraging VR technology for training delivery and evaluation can enhance accessibility and effectiveness, particularly in the context of remote work and digital IT trends. Moreover, this research underscores the importance of ongoing evaluation and adaptation of training programs to ensure relevance and impact. By soliciting feedback from employees and leveraging VR data analytics, ITs can refine their training strategies to address emerging challenges and capitalize on opportunities for improvement. Furthermore, compliance with regulatory requirements remains a crucial aspect of training and development efforts, necessitating proactive measures to stay abreast of industry regulations and best practices.

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**Table.1 Gender of the Employees**

| S.No | Gender Category | Respondent | Percentage |
|---|---|---|---|
| 1 | Male | 62 | 62 |
| 2 | Female | 38 | 38 |
| Total | 100 | 100 | |

**Source: Primary Data**

It is inferred from the Table 1 that 62% percent of employee category is male and 38% percent is female. It is analyses that under the demographic profile the consideration presents a comprehensive overview of the attributes of employees at a IT Sector.

**Table 2 Training and Development**

| S.No | Description | *SD* | *DA* | *NE* | *AG* | *SA* | *Mean* | *SD* |
|---|---|---|---|---|---|---|---|---|
| 1 | Employs competent and simulate real-life situations in training | 6.4 | 19.1 | 17.4 | 31.8 | 25.4 | 3.50 | 1.23 |
| 2 | Training is provided in traditional or VR mode. | 3.8 | 19.5 | 23.7 | 27.5 | 25.4 | 3.51 | 1.17 |
| 3 | Training increases your performance level with new techniques(Virtual Reality) | 4.7 | 18.0 | 20.1 | 29.7 | 27.5 | 3.57 | 1.19 |
| 4 | Training opportunities are available to everyone | 4.2 | 16.9 | 21.6 | 31.8 | 25.4 | 3.57 | 1.16 |
| 5 | Advanced level of training using VR can enhance your career | 5.3 | 15.9 | 20.6 | 32.8 | 25.4 | 3.57 | 1.17 |

**Source :Primary Data**

SD: Strongly Disagree; DA: Disagree; NE: Neither Agree nor Disagree; AG: Agree; SA: Strongly Agree; Mean .

The table 1 scrutinizes the perceptions of IT sector employees regarding the virtual training with the prevailing traditional training in the organization.

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**RESEARCH ARTICLE**

# Estimating the Approximate Solution of HIV Infection Model using Homotopy Perturbation Method (HPM)

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**ABSTRACT**

The current work uses the Homotopy Perturbation Method (HPM) to approximate the fundamental HIV viral dynamic model and describe the dynamics of the virus in a vulnerable population. Additionally, we evaluated the HPM on many implementations, demonstrating the correctness and efficiency of this approach. The estimated HPM solutions for the examined issues show good agreement with the Runge-Kutta method's numerical results.

**Keywords**: HPM,SIRC model;
**AMS Subject Classification:**34A34, 34G20, 46N60.

## INTRODUCTION

Virus infection involves free virions binding to target cells, entering, replicating, releasing multiple copies into the extracellular environment, and subsequently infecting nearby cells in a recursive process [1]. In the classical cell-free infection mode, virions move through the body via freely circulating particles, traversing cells and tissues through fluid phase diffusion, underscoring the significance of this infection route [2]. Contemporary infectious disease epidemiology heavily relies on computational model-based methods. Human immune deficiency virus (HIV), a retrovirus targeting CD4+T cells and macrophages, causes a progressive decline in the individual's immune system, typically culminating in AIDS within 10–15 years of initial infection. HIV transmission encompasses various routes, including contaminated blood transfusions, sharing needles, unprotected sex, childbirth, and breastfeeding [3]. The detrimental effects of HIV on the body result from its biological mechanisms and its interaction with the immune system of human. Regrettably, HIV infection causes the immune system to malfunction.An epidemic model that

exclusively focused on CTL was provided by Nowak and Bangham [4]. Additionally, a model of the Hepatitis C virus that takes into account both humoral and cell-mediated responses was expressed by Wodarz [5].A thorough examination of mathematical frameworks related to HIV pathogenesis during therapywascarriedout by Wodarz and Nowak [6].

Perelson and Ribeiro [7] scrutinized models elucidating the within-host dynamics of HIV infection.Researchers have regularly offered effective mathematical methods to address the CD4+T cell HIV infection model[8–12]. Using the Bessel collocation approach, Yuzbasi was able to derive numerical solutions for the HIV model of CD4+T cells [13].Karaçayır and Yuzbasi [14] applied a Galerkin method to address a epidemic model on HIV infection. Their study delved into the technique of residual correction, aiming to reduce approximation errors. Balamuralitharan and Geethamalini was applied a HPM method for solving Analytical solutions of the deterministic SEIA worm model [15–19]. In 1989, Perelson [20] developed a basic model with three variables that describes how HIV infects the human immune system:

Susceptible cells, infected cells, and virus. By adding four factors, Perelson et al. [21] enhanced this model and built on the groundwork established in their previous research.Uninfected cells denoted as X(t), Y(t) denotes infected cells and Z(t) denotes virus. The model equations are detailed in [6, 7, 20–24].

$$\begin{aligned} \frac{dx}{dt} &= \mu - \eta x - \varepsilon xz \\ \frac{dy}{dt} &= \varepsilon xz - (\rho + \sigma) y \\ \frac{dz}{dt} &= \chi y - \lambda z \end{aligned} \tag{1}$$

With $x(0) = N_1, y(0) = N_2, z(0) = N_3$.

## MATERIALS AND METHODS

Consider the equation:

$$A_1(\bar{u}) - f_1(\bar{r}_1) = 0,\ \bar{r}_1 \in \Omega, \tag{2}$$

subject to boundary condition

$$B_1(\bar{u}, \partial\bar{u} / \partial\bar{n}) = 0,\ \bar{r}_1 \in \Gamma, \tag{3}$$

In broad terms, one can decompose the operator A into two distinct components: a linear portion denoted as L and a nonlinear component represented as N.

$$\bar{L}_1(v_1) + \bar{N}_1(v_1) - f_1(\bar{r}_1) = 0. \tag{4}$$

Then Eq.(2) $v_1(\bar{r}_1, p) : \Omega \times [0,1] \to R$

$$H(v_1, p) = (1-p)[L_1(v_1) - L_1(u_0)] + p[A_1(v_1) - f_1(\bar{r}_1)] = 0,\ p \in [0,1], \bar{r}_1 \in \Omega \tag{5}$$

Which is

$$H(v_1, p) = L_1(v_1) - L_1(\bar{u}_0) + pL(\bar{u}_0) + p[N_1(v_1) - f_1(\bar{r}_1)] = 0 \tag{6}$$

The parameter p, which lies in the interval [0, 1]. This meets the requirements for the boundaries. From (5) and (6), it is evident that

$$H(v_1, 0) = L_1(v_1) - L(\bar{u}_0) = 0, H(v_1, 1) = A_1(v_1) - f_1(\bar{r}_1) = 0 \tag{7}$$

In this instance, the embedding parameter is seamlessly introduced, devoid of any artificial influences. Moreover, it can be regarded as a diminutive parameter within the range of 0 to 1. Then

$$v = v_0 + pv_1 + p^2 v_2 + \ldots \qquad (8)$$

Then,

$u = \lim_{p \to 1} v = v_0 + v_1 + v_2 + v_3 + \ldots$ (9) Theconvergenceofseries(9)hasbeendemonstratedbyHe[25].

## RESULTS AND DISCUSSION

Following the principles [26, 28-32]of the HPM, a homotopy for (1) can be formulated in the following manner:

$$p(\dot{v}_1 - \eta v_1 - \mu + \varepsilon v_1 v_3) + (1-p)(\dot{v}_1 - \dot{x}_0) = 0$$

$$p(\dot{v}_2 - \varepsilon v_1 v_3 + (\sigma + \rho) v_2) + (1-p)(\dot{v}_2 - \dot{y}_0) = 0 \qquad (10)$$

$$(1-p)(\dot{v}_3 - \dot{z}_0) + p(\dot{v}_3 + \lambda v_3 - \chi v_2) = 0$$

Where the first approximations are as follows, and dot stands for differentiation with respect to t:

$$\begin{aligned} v_{1,0}(t) &= x_0(t) = x(0) = N_1 \\ v_{2,0}(t) &= y_0(t) = y(0) = N_2 \\ v_{3,0}(t) &= z_0(t) = z(0) = N_3 \end{aligned} \qquad (11)$$

and

$$\begin{aligned} v_1 &= v_{1,0} + pv_{1,1} + p^2 v_{1,2} + p^3 v_{1,3} + \ldots \\ v_1 &= v_{1,0} + pv_{1,1} + p^2 v_{1,2} + p^3 v_{1,3} + \ldots \\ v_2 &= v_{2,0} + pv_{2,1} + p^2 v_{2,2} + p^3 v_{2,3} + \ldots \\ v_3 &= v_{3,0} + pv_{3,1} + p^2 v_{3,2} + p^3 v_{3,3} + \ldots \\ v_3 &= v_{3,0} + pv_{3,1} + p^2 v_{3,2} + p^3 v_{3,3} + \ldots \end{aligned} \qquad (12)$$

From equations (11) and (12) are substituted into Equation (10), then we have

$$\begin{aligned} & p(\dot{v}_{1,1} - \mu + \eta v_{1,0} + \varepsilon v_{1,0} v_{3,0}) + p^2(\dot{v}_{12} + \eta v_{1,1} + \varepsilon v_{1,0} v_{3,1} + \varepsilon v_{1,1} v_{3,0}) + \\ & p^3(\dot{v}_{13} + \eta v_{1,2} + \varepsilon v_{1,0} v_{3,2} + \varepsilon v_{1,1} v_{3,1} + \varepsilon v_{1,2} v_{3,0}) + \ldots = 0 \\ & p(v'_{2,1} - \varepsilon v_{1,0} v_{3,0} + \rho v_{2,0} + \sigma v_{2,0}) + p^2(v'_{2,2} - \varepsilon v_{1,0} v_{3,1} + \varepsilon v_{1,1} v_{3,0} + \rho v_{2,1} + \sigma v_{2,1}) + \\ & p^3(v'_{2,3} - \varepsilon v_{1,0} v_{3,2} - \varepsilon v_{1,1} v_{3,1} - \varepsilon v_{1,2} v_{3,0} + \rho v_{2,2} + \sigma v_{2,2}) + \ldots = 0 \\ & p(\dot{v}_{3,1} - \chi v_{2,0} + \lambda v_{3,0}) + p^2(\dot{v}_{32} - \chi v_{2,1} + \lambda v_{3,1}) + \\ & p^3(\dot{v}_{33} - \chi v_{2,2} + \lambda v_{3,2}) + \ldots = 0 \end{aligned} \qquad (13)$$

$$\dot{v}_{1,1} - \mu + \eta v_{1,0} + \varepsilon N_1 N_3 = 0$$

$$\dot{v}_{12} + \eta v_{1,1} + \varepsilon N_1 v_{3,1} + \varepsilon v_{1,1} N_3 = 0$$

$$\dot{v}_{13} + \eta v_{1,2} + \varepsilon N_1 v_{3,2} + \varepsilon v_{1,1} v_{3,1} + \varepsilon v_{1,2} N_3 = 0$$

$$\begin{aligned}
&\dot{v}_{2,1} - \varepsilon N_1 N_3 + \rho N_2 + \sigma N_2 = 0 \\
&\dot{v}_{2,2} - \varepsilon N_1 v_{3,1} + \varepsilon v_{1,1} N_3 + \rho v_{2,1} + \sigma v_{2,1} = 0 \\
&\dot{v}_{2,3} - \varepsilon N_1 v_{3,2} - \varepsilon v_{1,1} v_{3,1} - \varepsilon v_{1,2} N_3 + \rho v_{2,2} + \sigma v_{2,2} = 0 \\
&\dot{v}_{3,1} - \chi N_2 + \lambda N_3 = 0 \\
&\dot{v}_{3,2} - \chi v_{2,1} + \lambda v_{3,1} = 0 \\
&\dot{v}_{3,3} - \chi v_{2,2} + \lambda v_{3,2} = 0
\end{aligned} \tag{14}$$

$$\begin{aligned}
&x(t) = \lim_{p\to 1} v_1(t) = \sum_{k=0}^{k=3} v_{1,k}(t) \\
&y(t) = \lim_{p\to 1} v_2(t) = \sum_{k=0}^{k=3} v_{2,k}(t) \\
&z(t) = \lim_{p\to 1} v_3(t) = \sum_{k=0}^{k=3} v_{3,k}(t)
\end{aligned} \tag{15}$$

Therefore,

$$\begin{aligned}
&x(t) = N_1 + t(\mu - \eta N_1 - \varepsilon N_1 N_3) + \frac{t^2}{2}[(\mu - \eta N_1 - \varepsilon N_1 N_3)(-\eta - \varepsilon N_3) - \varepsilon N_1(\chi N_2 - \lambda N_3)] \\
&+\frac{t^3}{6}[((\mu - \eta N_1 - \varepsilon N_1 N_3)(-\eta - \varepsilon N_3) - \varepsilon N_1(\chi N_2 - \lambda N_3))(-\eta - \varepsilon N_3) - \\
&\varepsilon(N_1(\varepsilon\chi N_1 N_3 - \rho\chi N_2 - \sigma\chi N_2) - \lambda(\chi N_2 - \lambda N_3) + (\mu - \eta N_1 - \varepsilon N_1 N_3)(\chi N_2 - \lambda N_3))] \\
&y(t) = N_2 + t(\varepsilon N_1 N_3 - \rho N_2 - \sigma N_2) + \frac{t^2}{2}[\varepsilon(N_1(\chi N_2 - \lambda N_3) - (\mu - \eta N_1 - \varepsilon N_1 N_3)N_3) \\
&-(\varepsilon N_1 N_3 - \rho N_2 - \sigma N_2)(\rho + \sigma)] + \frac{t^3}{6}[\varepsilon[N_1(\chi(\varepsilon N_1 N_3 - \rho N_2 - \sigma N_2) - \lambda(\chi N_2 - \lambda N_3)) \\
&+(\mu - \eta N_1 - \varepsilon N_1 N_3)(\chi N_2 - \lambda N_3) + ((\mu - \eta N_1 - \varepsilon N_1 N_3)(-\eta - \varepsilon N_3) - \varepsilon N_1(\chi N_2 - \lambda N_3))N_3] \\
&-[\varepsilon N_1(\chi N_2 - \lambda N_3) - (\mu - \eta N_1 - \varepsilon N_1 N_3)N_3) - (\varepsilon N_1 N_3 - \rho N_2 - \sigma N_2)(\rho + \sigma)](\rho + \sigma)] \\
&z(t) = N_3 + t(\chi N_2 - \lambda N_3) + \frac{t^2}{2}[\chi(\varepsilon N_1 N_3 - \rho N_2 - \sigma N_3) - \lambda(\chi N_2 - \lambda N_3)] \\
&+\frac{t^3}{6}[\chi[\varepsilon(N_1(\chi N_2 - \lambda N_3) - (\mu - \eta N_1 - \varepsilon N_1 N_3)N_3) - (\varepsilon N_1 N_3 - \rho N_2 - \sigma N_2)(\rho + \sigma)] \\
&-\lambda[\chi(\varepsilon N_1 N_3 - \rho N_2 - \sigma N_2) - \lambda(\chi N_2 - \lambda N_3)]]
\end{aligned} \tag{16}$$

## NUMERICAL SIMULATIONS

In order to compare the findings with those of Biazar [27], the following parameter values are taken into account:

N1=20 Initial x(t) population, which are susceptible
N2=15 Initial y(t) population, which are infective
N3=10 Initial z(t) population, which are immune

β=0.01 Rate at which the susceptible population to become infected
γ=0.02 Rate at which the infective to immune population

Three term Approximations are computed and shown for x(t), y(t), and z(t)in Fig.2,3 &4. We obtained the following graphs using MATLAB.

$$x(t) = 20 - 3t - 0.045t^2 + 0.02805t^3$$
$$y(t) = 15 + 2.7t + 0.018t^2 - 0.02817t^3 \qquad (17)$$
$$z(t) = 10 + 0.3t + 0.07t^2 - 0.00012t^3$$

A comparison between the results demonstrates that the approximations of three terms' results of the HPM is same as the results of Fourth order Runge-Kutta method (RK4).

## CONCLUSION

The epidemic model's nonlinear system of differential equations can be solved using the HPM, which looks to be a very simple process and produces accurate and consistent results. When compared to the fourth-order Runge-Kutta method (RK4), less computing is required.

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**Table1. Parameters Meaning of HIV Infection**

| Parameters and functions | Meaning | Values |
|---|---|---|
| $x$ | Susceptible | Variable |
| $y$ | Infected | Variable |
| $z$ | Virus | Variable |
| $\mu$ | target cells | 10 cells |
| $\varepsilon$ | rate of Infection | 0.000024 $mm^3 day^{-1}$ |
| $\eta$ | Death amount for susceptible | 0.01 $day^{-1}$ |
| $\chi$ | virus production | 467 virions $cells^{-1}$ [33] |
| $\lambda$ | Amount of virus clearance | 3.4 $day^{-1}$ [33] |
| $\sigma$ | Death amount due to virus | 0.16 [33] |
| $\rho$ | Mortality rate of infected cells killed by CTLs | 0.01 $day^{-1}\ cell^{-1}$ [33] |

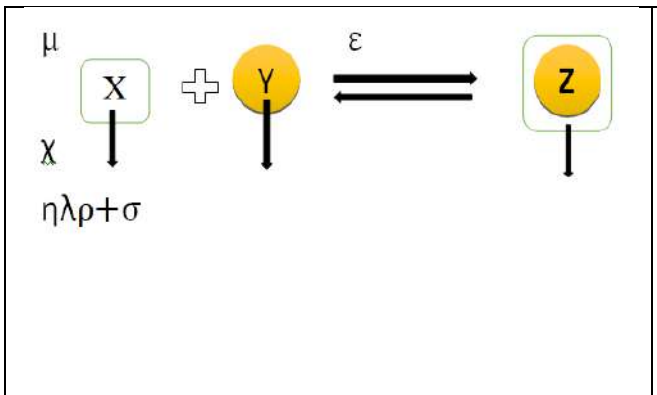


**Fig. 1. Basic HIV viral dynamics schematic diagram**

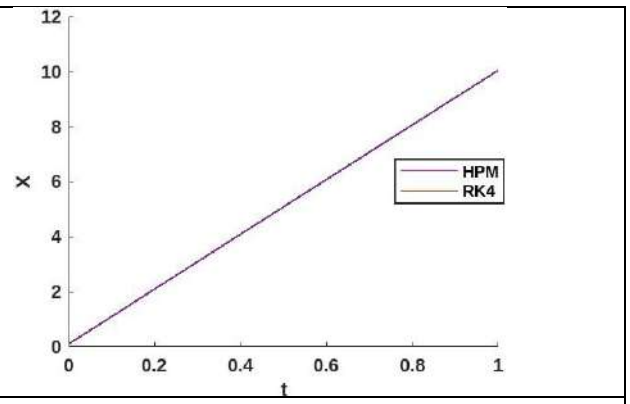


**Fig. 2. Approximations of three terms plotted against time for X(t)**

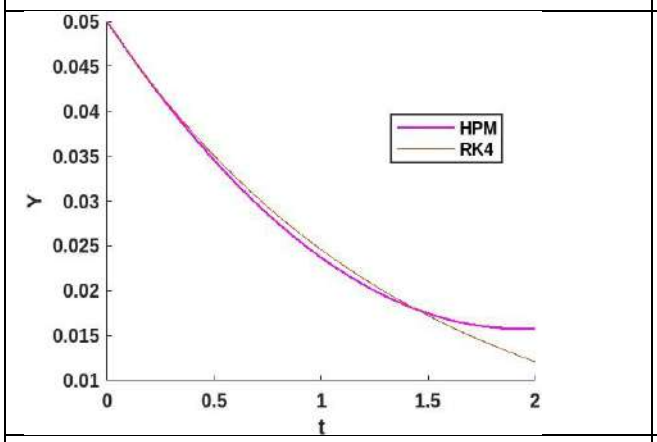


**Fig. 3. Approximations of three terms plotted against time for Y(t)**

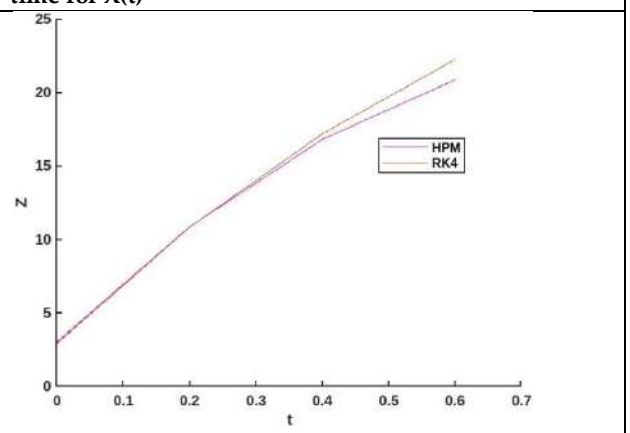


**Fig. 4 Approximations of three terms plotted against time for Z(t)**

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**REVIEW ARTICLE**

# Impact of Silver Nanoparticles against Hepatitis B Virus

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**ABSTRACT**

It has been shown that silver nanoparticles possess potentially cytoprotective effects against infected T-cells. However, the effects of these nanoparticles on other viruses are mostly unknown. This literature review examined the effects of silver nanoparticles on the hepatitis B virus (HBV). Emerging viral illnesses present enormous risks to public health throughout the whole world. This is particularly true in the wake of the COVID-19 epidemic, which was responsible for a considerable number of fatalities among humans and had severe repercussions on the global economy. It is noteworthy to note that studies have shown that silver nanoparticles, which are sometimes referred to as AgNPs, are capable of destroying viruses, bacteria, and fungi via a number of different approaches. This is a significant challenge for the pharmaceutical industry. It is difficult to create treatments that are effective against viruses yet do not cause harm to the cells of the host organism they are being used against. In recent years, there has been a great focus put on the exploration of how AgNPs interact with viruses. This study has been carried out in a number of different laboratories throughout the world. This article explores the prospect of using silver nanoparticles, which have antiviral properties, in future medical treatments for viral infections. The primary emphasis of this research is on the properties of silver nanoparticles (AgNPs), namely their antiviral activity, mechanisms, applications, and toxicity. Methodologies that have been used in the past to describe AgNPs are included in these features.

**Keywords:** silver nanoparticles, antiviral mechanism, hepatitis B, cytoprotective, viral infection

## INTRODUCTION

Silver nanoparticles, commonly known as AgNPs, have been the focus of much study into their antibacterial qualities due to the unique physical and chemical characteristics they possess. Because various kinds of hepatitis may result in infections and other health issues in individuals all over the world, hepatitis viruses continue to be a significant worldwide public health concern. Hepatitis viruses come in five distinct varieties, each identified by the letters A, B,

C, and D. Hepatitis viruses may transfer from person to person in a variety of ways, with various clinical symptoms and potential long-term health effects for patients. It is crucial to remember that although some research indicates silver nanoparticles may be able to stop viruses from multiplying, the information is still preliminary and cannot be taken as completely conclusive. Throughout human history, viruses have been responsible for many devastating pandemics. It is hypothesized that modern viruses originated in the same pre-existing cell populations as modern cells [1]. They may serve as vectors in a wide variety of organisms, including mammals, plants, bacteria, and fungi [2], and can be found in a size range from 20 to 900 nanometers. They may also take on a variety of chemical forms. Despite the widespread availability of hepatitis, A and B vaccines, it is essential to keep in mind that hepatitis C has no cure at the present time. The variety of therapies for chronic hepatitis C, however, has expanded greatly in recent years.

Emerging hepatitis virus infections can be caused by a variety of conditions, such as the emergence of novel strains or genotypes of the virus, rising transmission rates in some areas, changes to the disease's clinical presentation, or difficulties controlling the virus' spread. It is conceivable for viruses to spread from one person to another or from a mother to her offspring [3]. Some viruses may only be communicated by sexual contact, while others can spread through ingestion of contaminated liquids or foods. They may also be passed from person to person by saliva, coughing, sneezing, and other bodily fluids [3]. They may also be spread by kissing, spitting, and sneezing. Sexual contact is necessary for the spread of certain viruses, whereas contaminated water or food may spread others [3]. Additionally, it is known that certain viruses, such the ones that cause hepatitis or the human immunodeficiency virus (HIV), may induce recurrent infections that might lead to the growth of tumors or the development of acquired immunodeficiency. The prevalence of viral infections, their clinical significance, and the need for antiviral medications are all correlated [4]. Injurious organisms, such as viruses, are to blame for a significant portion of illness cases and fatalities worldwide. For instance, it has been calculated that over 2 million people worldwide die each year from viruses [5]. The highly contagious nature of these illnesses and the lack of effective management methods have serious negative effects on public health [6]. Numerous viral diseases, including smallpox, have been wiped out with the aid of vaccine campaigns. One of these viruses, smallpox, was eliminated in 1979 [7]. Numerous vaccines against viruses have been produced in recent years; nevertheless, additional shots and medications are still needed to lessen the impact [8,9].

Nanotechnology has enabled significant advancements in the fight against viruses during the last several years. Innovative biomolecular systems have been created as a result of the use of nanotechnology in biomedicine. These systems have the ability to differentiate between certain cell types, viruses, bacteria, and fungus. To do this, nanoscale (100 nm) characteristics are developed for individual components. The physical (plasmonic resonance and fluorescence amplification) and chemical features of nanoparticles have made them useful as antiviral agents. These skills are generated by a large number of surface atoms and a large surface area relative to total volume. When comparing nanoparticles to bulk materials or ions, important nanoparticle properties improve as particle diameter decreases. This is due to the fact that decreasing a particle's diameter significantly increases the accessible surface area of the particle. These characteristics pave the way for improved and safer drugs, targeted tissue therapies, individualized nanomedicines, and preventative health screenings [10]. Since nanoparticles are unique among chemicals in terms of their physical and chemical characteristics, scientists have been investigating whether or not they may halt viral infections. Among the many viruses that may cause hepatitis, the most well-known are Hepatitis B (HBV) and Hepatitis C (HCV). The identification of several nano-based compounds has enhanced both the efficacy of treatment and the targeted administration of antiviral medicines [11].

In this work, the antiviral efficacy of AgNPs against the hepatitis virus is reviewed, and the probable antiviral mechanisms underlying this activity are discussed. In addition, the characterization methods, toxicity ranges, and prospective applications of AgNPs are examined in this work. Conclusion: There is some indication that silver nanoparticles may be antiviral against HBV; nevertheless, further study is required to prove their safety and usefulness in clinical settings, optimize their characteristics for maximal performance, and completely understand their mechanisms of action.

**Synthesis of Silver Nanoparticles**

Silver nanoparticles, commonly known as AgNPs, have emerged as significant agents in a number of scientific domains due to the unique characteristics they contain and the many applications they may be used in. The potential of several nanoscale miracles has been unleashed throughout time by the development of novel synthesis methods. This article explores the synthesis of silver nanoparticles, shedding light on their production processes, the uses for which they are most helpful, and the promise for future advancement. In its simplest form, AgNP synthesis may be broken down into top-down and bottom-up processes. This "top-down approach" involves a number of physical forces, such as ball milling (which uses mechanical energy), grinding, and crushing, to create metal nanoparticles (NPs) from bulk materials. Vapor condensation employs thermal energy, whereas the laser ablation and electrical arc discharge methods also use electricity [12]. These procedures enable the synthesis of pure nanoparticles without the use of any chemicals, and may yield NPs with sizes ranging from 10 to 100 nm. When making nanoparticles, one may use physical methods to ensure both high purity and a uniform particle size distribution. Even if there aren't any chemical reactions that might endanger people or the environment, the physical process still struggles to avoid agglomeration since neither stabilizers nor capping agents are readily accessible. You will need to employ complicated equipment and outside power sources to complete these processes. On the other hand, bottom-up approaches start with single molecules and use nucleation and growth processes to form more complex clusters [12,13]. It has been shown that bottom-up strategies are more effective than top-down ones.

Silver nanoparticles may directly alter viruses and their initial host cell interactions depending on their size, shape, functionality, and composition. Silver nanoparticles are antiviral, although their mechanism is unknown. Many studies show that AgNPs may inactivate viral particles. This is done by eliminating viral components such capsid structural proteins and envelope glycoproteins. After an hour of infection, AgNP-decorated silica hybrid composites (Ag30-SiO2) suppressed IFV-A. The virus envelope glycoproteins hemagglutinin (HA) and neuroamidase (NA) also bonded with these composites, greatly reducing their activity. After studying how Ag30-SiO2 composites interact with the virus' outer membrane, researchers found that MDCK cells may be less susceptible to IFV-A infection [14]. To limit HBV genome replication, AgNO3 was utilized to build AgNPs with average diameters of 10 and 50 nm in HEPES buffer [15]. TEM showed that AgNPs linked to HepAD38 cells to create HBV pgRNA. The utilization of nanoparticles as a possible antiviral use is an exciting idea, according to the findings of recent study. Early study has shown the efficacy of AgNPs, despite the fact that their method of action is not entirely known. In-depth research into their antiviral efficacy against certain viruses has shown that it is sufficient to justify pursuing this area of study in order to produce nano-treatments that are effective against a broad range of viruses [16].

The emergence of nanotechnology, which functions on the microscopic scale, has resulted in a fundamental paradigm shift in the scientific world. Silver nanoparticles, which typically vary in size from 1 to 100 nanometers, exhibit distinct properties on the physical, chemical, and biological fronts as compared to bulk silver. This paves the way for several cutting-edge uses in electronics, medicine, catalysis, and other fields [16]. There are many techniques to create silver nanoparticles, each of which has benefits and may be customized to meet specific requirements, such as one of the following: The conventional method of reducing silver is called chemical reduction, and it calls for the employment of a reducing agent in addition to the reduction of a precursor silver salt. Salt is most often utilized in the form of sodium citrate or sodium borohydride. The next stage involves creating AgNPs utilizing physical methods such laser ablation, sputtering, and evaporation. These processes employ energy to the process, converting bulk silver into nanoparticles. Last but not least, a recently developed strategy called "Green Synthesis," which refers to nature-inspired synthesis methods and involves the use of plant extracts, microbes, or biomolecules as reducing and stabilizing agents, offers environmentally friendly routes to the production of AgNP [17]. The manufacture of silver nanoparticles is an interdisciplinary project that has drawn interest from researchers because it provides a window into the world of nanoscale phenomena. As science and technology advance, it will be possible to fully realize the promise of AgNPs in a range of applications by improving synthesis methods and understanding AgNP behavior. The investigation of the world at the nanoscale continues to change many industries and inspire previously unthinkable solutions.

**AgNPs Against Hepatitis B Virus**

Silver nanoparticles (AgNPs) and their impact on Hepatitis B virus (HBV) are now a research focus. Recent interest in silver nanoparticles (AgNPs) has been driven by the promise of their antibacterial effects, however studies examining the precise nature of AgNPs' interaction with HBV are still in their infancy. Antiviral drugs often prevent the production of new viruses by directly affecting the virus or by interfering with critical steps in the viral replication process [17]. In order to develop innovative antiviral therapies based on nanoparticles in a way that is precise and efficient, it is crucial to understand the antiviral mechanism of action of AgNPs. Although antiviral mechanisms are still being studied, it seems that AgNP production is a necessary component. Size, shape, and surface functionalization are some of the factors that affect antiviral efficacy [18,19].

As of 2007, six different medications have been approved for the treatment of HBV infection. Traditional interferon (IFN)-2b and pegylated IFN-2a are two examples of immunomodulatory drugs. Both of these are intended to aid the host immune system in regaining control of HBV, which should eventually lead to remission of the chronic condition without the need for medication. The other four medications work directly against viruses and are nucleoside analogues [20]. Lamivudine, adefovir, entecavir, and telbuvidine are these nucleoside analogues. IFN- may directly limit viral protein synthesis by regulating antiviral cytokines [21, 22]. IFN- may boost CD8+ cytotoxic T-lymphocyte and natural killer cell lysis of infected hepatocytes [21, 21]. Natural killer cells and CD8+ cytotoxic T lymphocytes can lyse infected hepatocytes faster [21]. AgNPs have the potential to be employed as a virucidal agent due to their capacity to alter the morphology of the virion and destroy the infectious viral particle. Additionally, by blocking the virus from adhering to the host cell or from entering cells, it may obstruct the first stages of viral reproduction. The inhibition of viral replication's late stages has also been proposed, although its effects within cells are less well understood [23]. Despite the fact that they are the antiviral mechanisms that are discussed the most often, this is the case.

From this perspective, a wide range of sectors have shown interest in AgNPs due to their broad-spectrum antiviral action as well as their ability to stop cell infection. The manufacturing of PPE, food packaging, textiles, and water and air purification systems are all examples of industries that are expected to develop rapidly in the next years. In reality, from a scientific perspective, the best cures would be those that either destroy viral particles or obstruct the attachment and entry activities of viruses. First, the capacity to avoid cell infection would lessen host toxicity and reduce the possibility of discovering virus resistance. Additionally, it would be useful for providing a quick and efficient strategy in combating newly emerging virus strains [23,24]. Some investigations have shown that AgNPs physically link to viruses, proving their antiviral properties. The findings of those investigations, however, merely suggested a possible method of action, suggesting the need for further investigation [25].

The ability of AgNPs to inhibit the replication of viruses is an intriguing trait that might be used in a variety of technologies. It may be feasible to utilize a variety of ways, such as coating with just AgNPs, altering the surface of the AgNPs themselves, or a combination of these and other methods, to prevent metallic nanoparticles from coming into direct contact with cells and maybe reduce their cytotoxicity. AgNPs that have been functionalized, coated, or integrated into a composite material may have their antiviral activity affected by the physical barriers created by the combination with another element. However, these strategies may also alter how viruses and nanoparticles interact. When metallic nanoparticles were encased in organic or inorganic materials like chitosan, collagen, or gelatin, the antiviral activity was much increased, and the impact lasted for a longer period of time because of the delayed ion release [26]. The nanoparticles' capacity to postpone ion release made this feasible.

This session will conclude with a discussion of the ideal qualities of a virucidal antiviral agent that is safe for humans and the environment. The composite system's antiviral activity was sensitive to AgNP size and concentration. Higher amounts and smaller AgNP dimensions increased the composite system's antiviral activity, whereas the chitosan matrix did not. The chitosan matrix, on the one hand, might help block the virus from attaching to the nanoparticles directly, or at least mitigate the virus's impact on them. However, since the matrix results in additional physical harm, virions attach to the composite material, which reduces their ability to interact with the host cells [27].

Although chitosan lacks inherent antiviral capabilities, it may significantly boost the efficacy of nanoparticles by acting as a matrix for them. The chitosan matrix and AgNPs combination falls within the same category. The influence that the precursor molecules and metabolites have, depending on the application, on the health and safety of either people or animals, or on the environment, must be examined and evaluated when selecting the system that will be employed [28]. The specific mechanism by which AgNPs eradicate viruses remains mostly unknown. AgNPs can protect against HSV, respiratory syncytial virus, and adenovirus type 3 [29]. Either avoiding viral infection in cells or directly inactivating viruses may achieve this.

The World Health Organization estimates that 887,000 individuals died in 2015 as a direct consequence of problems connected to HBV, such as cirrhosis and hepatocellular cancer. Some estimates put the number of persons infected with this virus at 227 million. The HBV core releases particles into liver cell nuclei, where they form covalently closed circular DNA (cccDNA) [30]. Virus-like particles, attenuated viruses, or protein-subunit antigens in vaccines activate the immune system and prevent illness. The preclinical stage of AgNP usage still has restrictions on its use. AgNPs' two main tactics for combating viruses that could be present in the human body are vaccination and oral delivery [31,32]. One of the best ways to save healthcare expenses while also lowering the chance of developing infectious diseases is to get vaccinated. Researchers have been examining the immunoactivity of both naturally occurring and synthetically manufactured nanoparticles since the development of nanotechnology [33,34]. Initial study reveals that silver nanoparticles (AgNPs) may have antiviral properties, but further research is needed, especially for hepatitis B virus. It is important to stress that the study is still in its early phases despite some data pointing to possible antiviral advantages of AgNPs. The optimal size, concentration, and surface qualities of AgNPs must also be determined via meticulous research in order to efficiently target HBV. Further research is required on the possible cytotoxicity and off-target effects of AgNPs on healthy cells. This is due to the possibility that nanoparticles may interact with a variety of biological elements other than the intended targets.

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**Conflict of Interest**
No conflict of interest

**Author's Contritbution**
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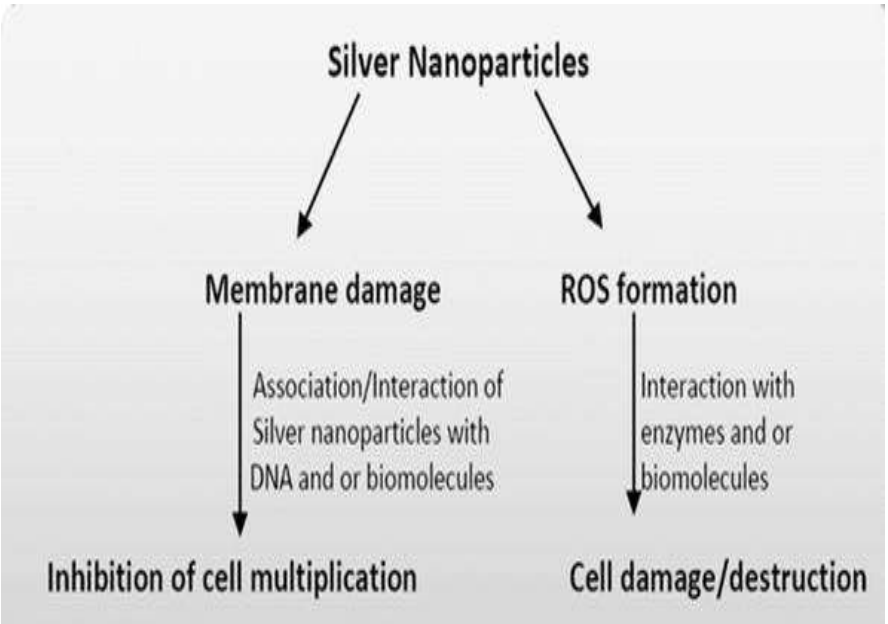


**Fig.1. Synthesis of Silver Nanoparticles**

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**RESEARCH ARTICLE**

# Screening of Colibactin Producing *Escherichia coli* from Urinary Isolates

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**ABSTRACT**

The study was conducted to characterize pathogenic *Escherichia coli (E. coli)* isolates from 35 patients with urinary tract infections (UTIs) and explore their genotoxinproducing potential, particularly focusing on the presence of the colibactin-producing gene, *clb*B. Biochemical tests confirmed the presence of *E. coli* in 22 isolates, which were then subjected to PCR analysis for the *clb*B gene. Out of the 22 isolates, 2 (9%) were found to harbor the *clb*Bgene, indicating the presence of colibactin-producing *E. coli*. The size of the amplicon generated with the primer set was consistent with previous studies. These findings suggest that colibactin-positive *E. coli* is present in UTI patients, potentially implicating its role in bladder cancer development. However, the study acknowledges its retrospective nature and limited patient information, emphasizing the importance of further research, including detailed comparative genomics analysis, to understand the role of colibactin-producing *E. coli* in bladder cancer and potentially prevent its incidence.

**Keywords:** *Escherichia coli* [*E. coli*], UTI, *clbB* gene, Bladder cancer, PCR.

## INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections, and *Escherichia coli (E. coli)* is the predominant bacterium responsible for these infections. A UTI refers to the presence of a certain number of bacteria in the urine (usually > $10^5$ /ml) [Foxman 2014; Smelov*et al.,* 2016]. UTI incidence in women is much higher than that in men [Erdem 2018].Three or more urinary tract infections within 12 months are defined as recurrent UTIs, as are two or more recurrences within six months. Relapses are usually caused by the same type of bacteria that caused the previous infection. 95% of all UTIs occur as ascending infections [Bacheller 1997].

*E. coli* strains of biological significance to humans may be broadly categorised as (1) commensal strains, (2) intestinal pathogenic strains and (3) extraintestinal pathogenic *E. coli* (ExPEC) strains. Among ExPEC, strains of

uropathogenic*E. coli* (UPEC) are most commonly associated with human disease. *E. coli* containing *pks* is relatively common bacterium found in the human gut and can be isolated from multiple sites of the human body [Fais *et al.,* 2018] and certain strains have been identified as producers of colibactin. The genotoxic chemical compound colibactin is synthesized by a complex machinery involving polyketide synthases (*PKS*), non-ribosomal peptide synthases (NRPS), and is 54 kilobases in size. The genotoxic nature of colibactin is due to its ability to induce DNA damage in host cells [Nougayrede*et al.,* 2006]. This genotoxin causes DNA double-strand breaks leading to genetic mutations, chromosomal abnormalities, and cell cycle arrest in the G2/M phase chromosomal instability, and premature aging, which ultimately lead to tumorigenesis [Cuevas-Ramos *et al.,* 2010; Secher *et al.,* 2013; Cougnoux*et al.,* 2014]. *pks* islands are widely distributed in group B2 *E. coli* strains isolated from intestinal or extraintestinal sites [Forough *et al.,* 2012]. Colibactin-producing bacteria cause DNA damage in bladder cells, including regenerating urothelial cells, and colibactin is produced by clinical *pks* UPEC strains isolated from human UTIs [Camille *et al.,* 2021]. Bladder cancer is a common malignancy, worldwide; it is the seventh most prevalent cancer, accounting for 3.2% of all malignancies [El-Mosalamy*et al.,* 2012]. There is paucity of studies on uropathogenic*E. coli* producing colibactin. The current study was designed to characterize pathogenic *E. coli* from isolates obtained from patients with UTIs and attempted to explore their colibactin producing potential.

## MATERIALS REQUIRED

Standard Glasswares, Nutrient agar plates, Eosin Methylene Blue agar plates, Gram's Staining reagents, Biochemical reagents, Solution A [10% SDS], Solution B [10mM Tris, 10mM MgCl2, 10mM KCl, 2mM EDTA, 0.4mM NaCl], Solution C [100µl phenol and 100µl chloroform], 1% agarose, TE and TBE buffers, Electrophoresis unit and UV Transilluminator.

## METHODOLOGY

A total of 34 *E. coli* isolates were obtained from urinary specimens of UTI specimens of UTI patients and transported to laboratory in aseptic condition for a further process. The collected strains were streaked and labelled on Nutrient agar plates and incubated at 37°C for 24 hours. Identification of *E. coli* was done by performing standard test and biochemical assays including gram staining, motility, IMViC were performed. Selected 2 – 3 well grown colonies with the same morphology were picked and streaked on to the surface of EMB agar. The plates are then incubated at 37°C for 24 hours. The growth and colony morphology were observed.

**Isolation of DNA and Agarose Gel Electrophoresis:**

DNA was isolated as per the standard procedure and the separated DNA samples, along with a molecular weight marker, were visualized under a UV Transilluminator after agarose gel electrophoresis according to the standard procedure. The DNA isolation technique led to efficient extraction with good quantity and quality of DNA, which was pure and devoid of contaminants, such as RNA and Proteins.

**PCR Amplification**

The DNA isolated from the *E. coli* isolates were further investigated for colibactin production. The primers used for the determination of *clb*+*E. coli* isolates [Hirayama *et al.,* 2019; Tsunematsu *et al.,* 2021] are shown below:

*clb*B-F:5′-TGTTCCGTTTTGTGTGGTTTCAGCG-3′ *clb*B-R: 5′-GTGCGCTGACCATTGAAGATTTCCG-3′

The primers used for the determination of *clb*+ *E. coli* isolates (*Clb*B). The PCR conditions used for colibactin genes amplification were as the initial denaturation was given at 95˚C for 10 minutes, denaturation at 95˚C for 15 seconds, annealing at 60˚C for 1 minute, extension at 72˚C for 40 seconds followed by the final extension at 72˚C for 7 minutes. Amplified products were visualized by agarose gel electrophoresis.

## RESULTS

A total of 34 isolates from urine sample were subcultured on nutrient agar out of these 31 gave positive for nutrient agar and 3 isolates failed to grow. [Fig.1]

Of the 31 isolates, 20 isolates gave positive for the following tests:
Gram Staining – Gram-Negative rods were observed. [Fig.2]Motility Test – it was observed to be motile.
Biochemical Analysis – it was observed positive for Indole and MR and negative for VP and Citrate test. [Fig.3]
Catalase Test – Positive [Fig.4]; Oxidase Test – Negative. [Fig.5]

Also, these strains showed positive result for catalase test [Fig.4] and negative result for oxidase test [Fig.5].
The 20 strains showed green metallic sheen in EMB [Fig.6] and positive for *E. coli* were picturized

The positive *E. coli* strains obtained from EMB plates were further subcultured on
Nutrient broth and DNA was isolated and was further confirmed by Agarose Gel Electrophoresis.

**Results of Agarose Gel Electrophoresis**
After electrophoresis, the gel was examined under UV light, and the DNA bands were observed [Fig.7]. The presence of bright bands at specific positions on the gel indicated the presence of DNA fragments.

**Detection of Colibactin Genes by PCR**

The extracted DNA was used as a template in PCR reactions, along with the designed primers and PCR reagents (polymerase, nucleotides, buffer). The PCR conditions (annealing temperature, cycle number, etc.) were optimized to ensure specific amplification of the target genes. The PCR products were separated by gel electrophoresis to confirm the presence and size of the amplified fragments. The presence of a band of the expected size indicated the presence of the target gene in the sample. The amplified product was detected to be around 450 basepair in size and it was indicated by the presence of band [Fig.8].

## DISCUSSION

Urinary tract infections (UTIs) are among the most commoninfections. *Escherichia coli* strains, termed uropathogenic*E. coli* (UPEC) are the most common causative agent in both uncomplicated and complicated UTIs causing approximately 80% of all UTIs. This biosynthetic gene encodes for a secondary metabolite named colibactin, putatively acquired through horizontal gene transfer as part of a mobile genetic element. The current study was designed to characterize pathogenic *E. coli* from isolates obtained from 35 patients with UTIs and explore their, genotoxin or colibactin producing potential. All the bacterial isolates were tested with a set of standard biochemical testsand their culture characteristics on MacConkey agar and nutrient agar and were identified based on colony morphology on nutrient agar, MacConkey's agar and EMB agar. 4–5 suspected colonies from nutrient agar bacterial plate were picked, cultured and then identified by the various biochemical tests. Biochemical tests were performed to confirm *E. coli* using Gram staining, catalase test, Indole, Methyl red, Voges-Proskauer test, Simmons citrate agar and various sugar fermentation tests. Bacterial samples with biochemical and culture characteristics consistent with those of *E. coli* were considered to be confirmed to be *E. coli* and evaluated in further testing.

*E. coli* infection might play a role in the development of bladder cancer. A study based on a rat model by El-Mosalamy*et al.,* 2012; Ashmawey*et al.,* 2011 and Chagneau*et al.,* 2021 showed that *E. coli* infection in the bladder tissues increases the carcinogenic ability and tumor enhancing effect of *E. coli*. UPEC strains which routinely cause infections have been shown to belong to phylogroups B2 and are often the same ones responsible for UTIs, but to date, a few studies has been conducted on colibactin and UTIs. The current study was initiated as a screening effort

to examine the prevalence of *clb* + *E. coli*. In the present study we attempted to analyse for the presence of colibactin producing gene *clb*B in the 20 of the 22 isolates which were biochemically confirmed as *E. coli.* DNA was extracted from the isolates. The extracted DNA was subjected to PCR andqualitatively analysed for *clb*B present in the DNA extract by amplifying the genefragmentusing a primer set. The primers were designed for the determination of colibactin-producing *E. coliClb*B gene. In the present study, we observed that 2 out of the 22 Uropathogenic *Escherichia coli* isolates harboured *clb* B *E. coli.* The size of the *clb*+ amplicon generated with the primer set *clb*-F/ *clb*-R is around 450 bp. This is in accordance with Tsunematsu *et al.,* 2021who used the same primers for amplification andreceived an amplicon size of 555bp andHirayama *et al*., 2019 who carried a PCR-based screening of the *E. coli* isolates for the presence of the *clb* gene cluster and demonstrated a 464-bp product. The search however did not identify *clb*B *E. coli* in remaining 18 isolates, as isolates without *clb* gene cluster would not yield anyproduct. We initiated a screening effort to examine the prevalence of *clb*+ *E. coli* among individuals with UTI. Screening of *E. coli* isolates led to the isolation of colibactin producer *E. coli*. Our study confirms the occurrence of *pks*+ UPEC. Our findings indicate that colibactinpositive *E. coli* appears to be produced in humans, by detecting it in the urine isolates obtained from patients suffering from urinary tract infections. Hence phenotypical and genotypical characters of the infecting *E. coli* virulent strains need to be done which would be a possibility of preventing bladder cancer at early stages.

## CONCLUSION

Our findings indicate that virulent strains capable of causing UTI harbour*clbB*+ gene. Here, we show that this genotoxin is produced in humans, by detecting it in the isolates obtained from urine of patients suffering from urinary tract infections. However, this study is a retrospective analysis and only a limited patient information has been collected. It will be worth checking the production of colibactin in all UTI isolates by detailed comparative genomics analysis for a better understanding of their role in bladder cancer. We conclude that to reduce the number of bladder cancer incidences and mortalities, it is vital to identify all the *E. coli* infection for *clb*B+ gene as it might play a role in the development of bladder cancer.

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**Fig: 1 – Growth of urinary isolates on Nutrient Agar**

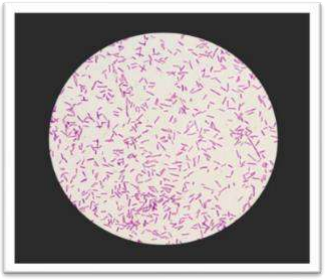

**Fig: 2 - Gram staining of *E. coli***

| | |
|---|---|
| Indole [A] | + |
| Methyl Red [B] | + |
| Vogues Proskauer [C] | - |
| Citrate Utilization test [D] | - |

**Table: 3 – Biochemical analysis**

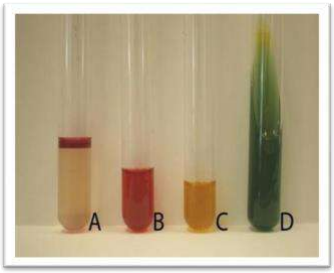


**Fig: 4 – Biochemical analysis of *E. coli***

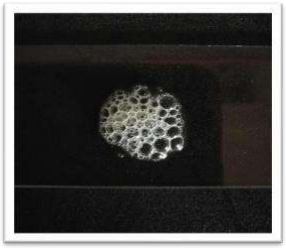

**Fig: 5 - Catalase Positive**

**Fig: 6 - Oxidase Negative**

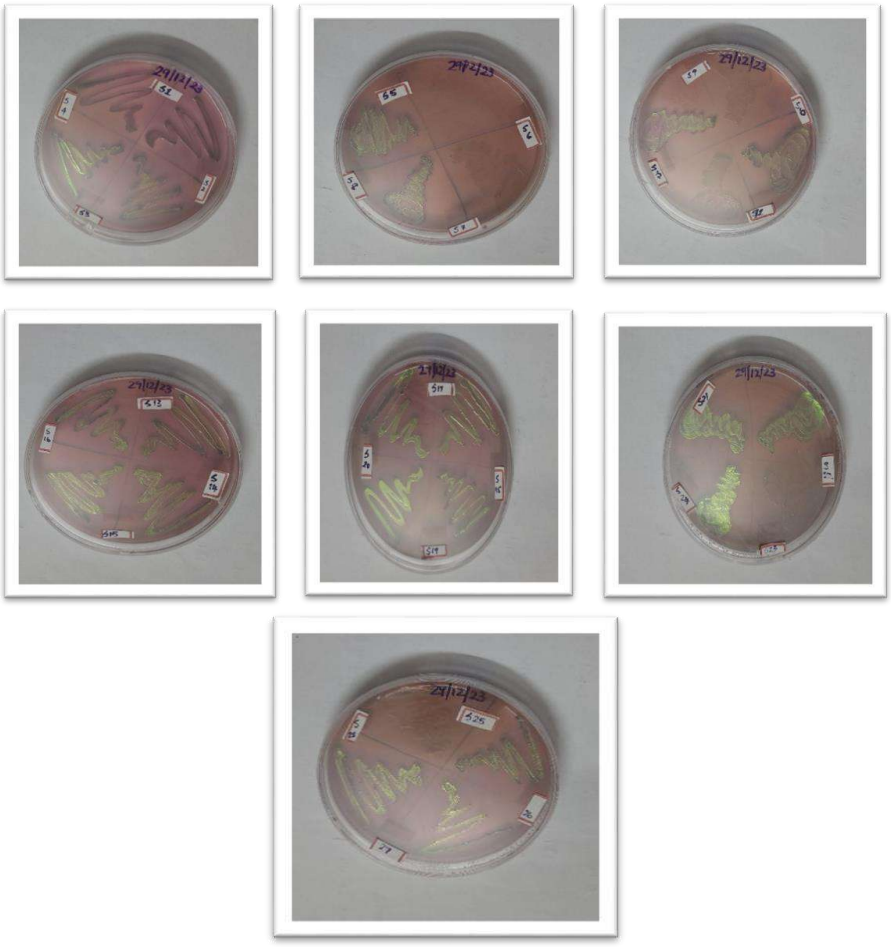

**Fig: 7 - *E. coli* isolates showing metallic sheen on EMB agar**

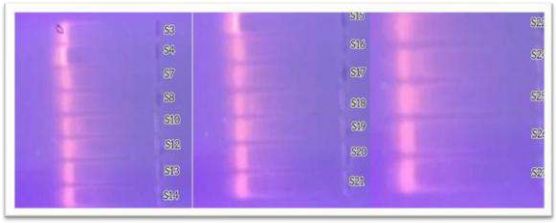

**Fig: 7 - DNA bands on agarose gel electrophoresis**

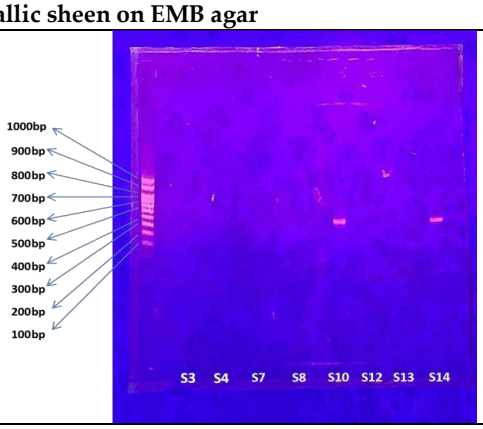


**Fig: 8 - Polymerase chain reaction assays for colibactin gene. Lane 1: Standard control; Lane 2 to Lane 5: Strains 3, 4, 7, 8 showing negative for *clb*B gene; Lane 6: Strain 10 showing positive for *clb*B gene; Lane 7 and Lane 8: Strains 12 and 13 showing negative for *clb*B gene; Lane 9: Strain 14 showing positive for *clb*B gene;**

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**RESEARCH ARTICLE**

# Cardioprotective Activity of *Hylocereus undantus* Fruit Peel Extract against Cyclophosphamide Induced Cardiotoxicity in Rats

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## ABSTRACT

Cardioprotective activity of *Hylocereus undantus* fruit peel extract against Cyclophosphamide induced Cardiotoxicity in rats. Cardiotoxicity was induced in Wistar rats by administering single injection cyclophosphamide (150 mg/kg) intraperitoneally on first day of experimental period. *Hylocereus undantus* (250 and 500 mg/kg, p.o.) was administered for 11 days after the single dose administration of cyclophosphamide (150 mg/kg) intraperitoneally. Plasma parameters, Cardiac biomarkers, pro-oxidant and antioxidant parameters and Histopathological Examinations were measured. Cyclophosphamide administration significantly increased lipid peroxidation and decreased the levels of antioxidant markers such as reduced superoxide dismutase and catalase. Cyclophosphamide elevated the levels of biomarker enzymes creatine kinase isoenzyme MB, lactate dehydrogenase, aspartate transaminase, alanine transaminase and alkaline phosphatase and reduced Total protein levels. Treatment with *Hylocereus undantus* extract significantly reversed the status of altered plasma biochemical parameters, cardiac biomarkers and oxidative enzymes in cyclophosphamide induced cardiotoxicity. Potential cardioprotective effect of *Hylocereus undantus* was supported by histopathological examination that reduced severity of cellular damage of the myocardial fibres. The biochemical, cardiac and histopathology reports support the cardioprotective effect of *Hylocereus undantus* which could be attributed to antioxidant activity.

**Keywords:** Cardioprotective; *Hylocereus undantus*; Cyclophosphamide; Oxidative stress; Myocardial infarction.

## INTRODUCTION

Cardiovascular disease (CVD) is increasingly recognized as the leading cause of death. The epidemiological change of the twentieth century established cardiovascular disease as the primary cause of disability worldwide. Global health projections imply that it will be the major cause of death in 2030. CVD is a set of heart and blood vessel illnesses. It comprises coronary heart disease (CHD), peripheral arterial disease (PAD), various types of angina, congestive heart failure (CHF), and myocardial infarction (MI) [1]. Cardiac damage is a potential short- or long-term complication of various anticancer treatments, which increases morbidity and death significantly [2, 3]. However, as cancer survival rates have increased due to advances in therapy, late cardiovascular adverse effects have emerged as a significant management challenge, notably in children malignancies, lymphoma, leukemia, and breast cancer [4]. Endocardial lipid peroxidation is caused by the excessive production of reactive oxygen species (ROS). Such damage causes the loss of cardioprotective antioxidants, increased oxidative stress, and apoptosis [5]. Medicinal plants include several antioxidant chemicals that protect against a variety of ailments, making them potential alternative therapeutic ingredients. Herbal antioxidants are increasingly being used to protect against a variety of cardiovascular problems. Bioactive substances derived from natural sources have become increasingly important in modern medicine, lowering the risk of cardiac diseases by scavenging free radical production [6].

*Hylocereus undatus* (Haw.) Britton and Rose belong to the Cactaceae family. The fruit is sweet and juicy, measuring up to 7 to 14 cm long and 5 to 9 cm wide, with a white, red, or purple pulp and numerous small, black, shiny seeds [7]. The Mayas utilized the fruit as hypoglycaemic, diuretic, heart disease prevention, wound disinfecting, tumour dissolving with stem sap, and dysentery treatment. Natural colorants may be recovered from the pulp and peel, and they have a high potential for application as colorants in the food industry due to their stability throughout processing and storage. *Hylocereus undatus* has high levels of vitamin B1, B2, B3, and C, as well as protein, fat, carbohydrate, crude fibre, and other nutrients and minerals. Thiamine, niacin, pyridoxine, cobalamin, glucose, flavonoid betacyanins, polyphenols, carotenoids, phosphorus, phenolic phytoalbumin, and iron. The peel contains more flavonoids than the meat, and ethanolic extracts of H. undatus peel and flesh have been shown to have varying antioxidant capabilities, anti-cancer activity, antimicrobial activity, hypocholesterolemic effect, and prebiotic effect [8-12]. The present study was aimed at investigating the Cardioprotective activity of *Hylocereus undatus* fruit peel extract against Cyclophosphamide induced Cardiotoxicity in rats.

## MATERIALS AND METHODS

*Hylocereus undatus* fruit peels were collected from commercial fruit juice shops in the surrounding areas of Tirupati and authenticated by Dr. M. Niranjan Babu, Professor, Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh, India. The fruit peels were washed, chopped into little 2-3cm pieces, and shade dried at room temperature for 15 days. The shade-dried fruit peels were finely grounded using a dry grinder and was soaked in 70% hydroalcoholic solvent at a 1:4 ratio in a narrow mouthed bottle for seven days, shaking occasionally using the maceration process. The extract was filtered with Whattman filter paper, and the solvent was evaporated to dryness using a rotary evaporator [13, 14]. The hydroalcoholic extract of *Hylocereus undatus* (HAHU) was used for pharmacological screening of Cardioprotective activity.

**Animal Husbandry**

The current study used healthy adult male wistar rats measuring 200-235 grams. Rats were housed in polypropylene cages with a standardized 12-hour light/dark cycle, 24 ± 2°C temperature, and 35 to 60% humidity. They had access to a pellet food and filtered drinking water at all times. The animals were fasted 24 hours before the induction of cardiotoxicity, although they had free access to water during the experimental period.

**Experimentation**

Cardiotoxicity had been demonstrated in rats by administering a single dose of cyclophosphamide in normal saline (150 mg/kg, body weight) intraperitoneally [15]. Animals were divided into four groups of six each. Group I administered with 0.5mL of normal saline p.o. as the vehicle control. Group II had a single dose of cyclophosphamide in normal saline (150 mg/kg, b.wt.) intraperitoneally on Day 01 served as cardiotoxic control. Group III received hydroalcoholic extract of *Hylocereus undatus* (250 mg/ kg, b.wt. p.o.) and single dose of cyclophosphamide in normal saline (150 mg/kg, b.wt.) intraperitoneally served as HAHU-I. Group IV received hydroalcoholic extract of *Hylocereus undatus* (500 mg/ kg, b.wt. p.o.) and single dose of cyclophosphamide in normal saline (150 mg/kg, b.wt.) intraperitoneally served as HAHU-II. The total duration of the experimentation was carried out for 11 days.

On day 12, each animal's blood was extracted via retro-orbital route and centrifuged at 3000 rpm for 10 minutes to separate plasma. The Plasma samples were tested for Serum Glutamate Oxalate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), and total protein using commercially available kit through biochemistry analyser (Microlab 400) [16-19] and Cardiac Biomarkers Lactate dehydrogenase (LDH) and Creatine kinase isoenzyme MB (CK-MB) [20]. Catalase (CAT), Superoxide Dismutase (SOD) and Malondialdehyde (MDA) levels were measured for the pro-oxidant and antioxidant activity [21-23]. The heart tissues were isolated and immediately rinsed with saline and preserved in a 10% formalin solution. Following fixation, the heart tissues were treated in an alcohol-xylene series and embedded in paraffin. The serial sections were cut and stained with haematoxylin and eosin. The slides were viewed under a microscope, and images were taken.

## STATISTICAL ANALYSIS

The results were expressed as the mean ± SEM and analyzed using one way ANOVA followed by Dunnett`s comparison test with Cardiotoxic control. Data were computed for statistical analysis using the graph pad software (Prism 5.0 version).

## RESULTS

Figure 1: Effect of HAHU on plasma biochemical parameters in Cyclophosphamide induced Cardiotoxicity in rats Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with Cardiotoxicity control (One-way ANOVA followed by Dunnet's test). Figure 2: Effect of HAHU on plasma Total protein in Cyclophosphamide induced Cardiotoxicity in rats Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with Cardiotoxicity control (One-way ANOVA followed by Dunnet's test). Figure 3: Effect of HAHU on LDH and CK-MB parameters in Cyclophosphamide induced Cardiotoxicity in rats Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with Cardiotoxicity control (One-way ANOVA followed by Dunnet's test). Figure 4: Effect of HAHU on Pro-oxidant and antioxidant parameters in Cyclophosphamide induced Cardiotoxicity in rats Values were expressed as Mean ± SEM (n=6). *P<0.05,**P<0.01,***P<0.001.as compared with Cardiotoxicity control (One-way ANOVA followed by Dunnet's test).

## DISCUSSION

Cyclophosphamide is an alkylating agent used in cancer therapy [24]. It is commonly used to treat leukaemia's, lymphomas, multiple myeloma, rheumatoid arthritis, and to prepare for bone marrow transplantation [25]. Despite its several therapeutic uses, it is a cardiotoxic drug that promotes endothelial dysfunction and myocardial cell death [26]. Oxidative stress is a main cause of cyclophosphamide induced Cardiotoxicity [27]. Oxidative stress can lead to endothelial dysfunction, hypertrophy, fibrosis, inflammation, apoptosis, cell migration, and angiogenesis [28]. Cyclophosphamide induced cardiotoxicity is primarily caused by its toxic product, acrolein, which interacts with the

tissue antioxidant defense system and produces an excessive amount of reactive oxygen species [29]. There is currently no viable medication to alleviate the cardiotoxic effects of Cyclophosphamide. Developing alternate ways to protect against Cyclophosphamide induced cardiotoxicity will be of enormous therapeutic value. In addition to their traditional values, herbal medicines are highly sought after by the public and medical community globally as sources of innovative lead chemicals for pharmaceutical development. As a result, medicinal plants will serve as a natural defensive approach and will be readily available at a lower cost than synthetic medications [30]. The present study was aimed at investigating the Cardioprotective activity of *Hylocereus undatus* fruit peel extract against Cyclophosphamide induced Cardiotoxicity in rats.

The activity of the liver enzymes ALT, AST, ALP, and TP in plasma are reliable indicators of hepatic and cardiac damage [31]. Cyclophosphamide increases plasma levels of these enzymes, indicating cellular injury and loss of functional integrity cell membrane, resulting in their leaking into the serum or plasma [32-34]. AST is an enzyme that is prevalent in the liver's cytoplasm and mitochondria, as well as the heart, skeletal muscle and brain. ALT is a hepatospecific enzyme primarily present in the cytoplasm [35, 36]. ALP is associated with the cell membrane, and its rise in the plasma is an indicator of impaired intrahepatic and extrahepatic bile flow (cholestasis), hepatobiliary damage, and overproduction or leakage of ALP [37, 38]. From the result of this study, pre-treatment with HAHU at both dose levels restored the activities of ALT, AST, ALP and TP [39]. LDH is an important enzyme involved in energy production, and it is a good biomarker indicating cell damage [40]. Additionally, CK-MB is an enzyme found mostly in the heart and presents with significantly high amounts during cardiac damage. In this study, there was a considerable rise in LDH and CK-MB activity following single administration of cyclophosphamide compared to the control group, which reflects the severe cardiotoxicity of cyclophosphamide, as ensured by the results of earlier investigations. Cyclophosphamide is cardiotoxic because it causes endothelial dysfunction and damage cardiomyocytes. Furthermore, cyclophosphamide induces lipid peroxidation, which disrupts endothelial cell permeability, leading to increased serum LDH and CK-MB levels [41]. However, by the protective administration of HAHU with cyclophosphamide, serum levels of LDH and CK-MB significantly diminished compared with the cyclophosphamide treated group.

The current study found that rats treated with cyclophosphamide had significantly lower SOD and CAT activity, as well as higher MDA levels, when compared to the vehicle control group. It has been observed that free radicals generated during CP treatment cause membrane damage, resulting in the loss of function and integrity of the cardiac membrane [15]. Cyclophosphamide causes a considerable decrease in SOD and CAT activity, which promotes the production of OH radicals and the onset and propagation of lipid peroxidation. However, it is proposed that the decrease in the activity of antioxidant enzymes is the result of increased oxidative stress in the cardiac tissues due to the overproduction of active reactive oxygen species [42,43]. Treatment of Cyclophosphamide treated animals with HAHU restored the antioxidant enzymes (SOD and CAT) activities and decreased MDA levels in the cardiac tissues towards control level, indicating a protective effect of HAHU against reactive oxygen species. This action could be attributed to its ability to reduce oxidative stress and preserve the activity of antioxidant enzymes, as well as its ability to suppress lipid peroxidation hydroxyl radical [44].

Histopathological comes about of heart tissue in control rats heart tissue appeared intaglio myocardial strands and pericardium; whereas cyclophosphamide (CP) rats heart tissue exhibited enormous alter in the myocardium appearing a shifting degree of vacuolar changes in the cardiac muscle filaments, basically in the frame of degeneration of myocardial strands, vacuolization of the cardiomyocytes, penetration of provocative cells, myofibrillar damage, and hypertrophic myocardial fibre with irritation; HAHU-I (Low Dose test Extract) treated showed direct greasy modification in certain muscle cells and separation of a few cardiac strands; and HAHU-II (High Dose test extract) treated rats showed enhancement in histoarchitecture.

## CONCLUSION

The current study found that the hydroalcoholic extract of *Hyoclocereus undantus* fruit peels had strong Cardioprotective action in various parameters, which is dose dependant. The extract has the strongest protective impact against the negative effects of cyclophosphamide on plasma profiles, cardiac biomarkers, and oxidative stress parameters. The extract's antioxidant polyphenolic components may be responsible for its cardioprotective activity. However, chemical isolation is recommended in order to discover the precise phytoconstituents responsible for *Hyoclocereus undantus* crude extract's cardioprotective properties. More relevant models with direct heart injury and endogenous antioxidant enzymes should be used to support the finding. Furthermore, proteomic analysis is necessary to analyse protein expression levels.

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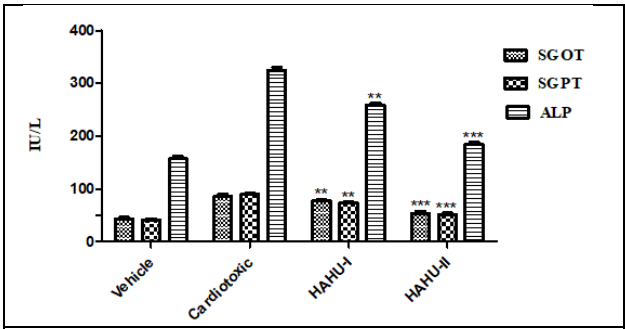


**Figure 1: Effect of HAHU on plasma biochemical parameters in Cyclophosphamide induced Cardiotoxicity in rats**

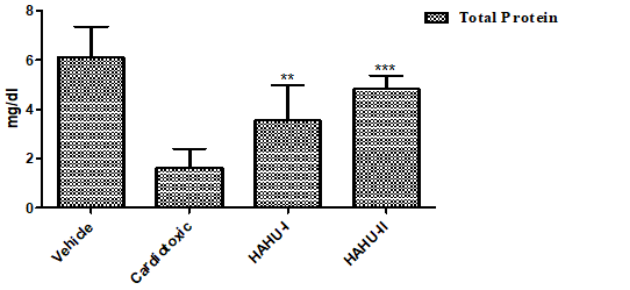


**Figure 2: Effect of HAHU on plasma Total protein in Cyclophosphamide induced Cardiotoxicity in rats**

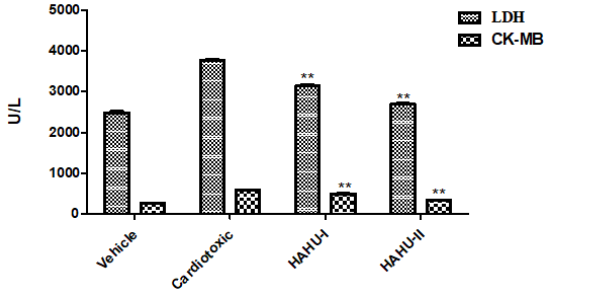


**Figure 3: Effect of HAHU on LDH and CK-MB parameters in Cyclophosphamide induced Cardiotoxicity in rats**

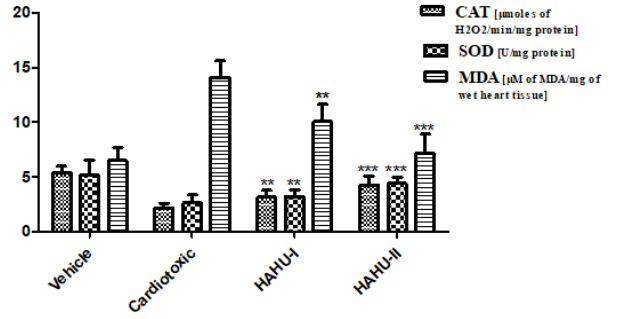


**Figure 4: Effect of HAHU on Pro-oxidant and antioxidant parameters in Cyclophosphamide induced Cardiotoxicity in rats**

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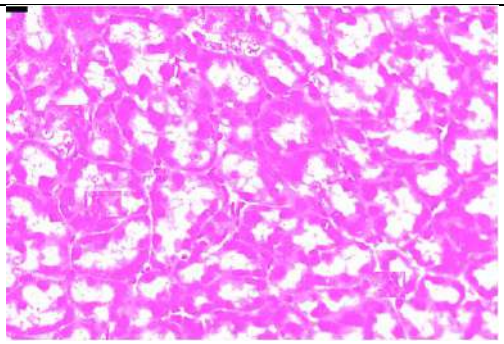
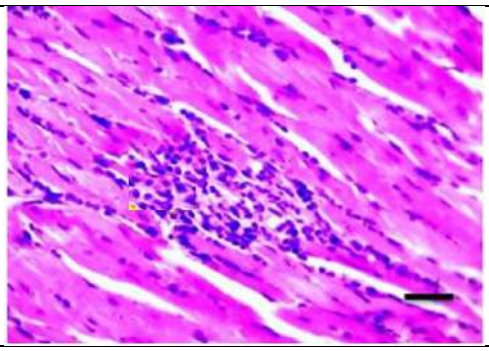
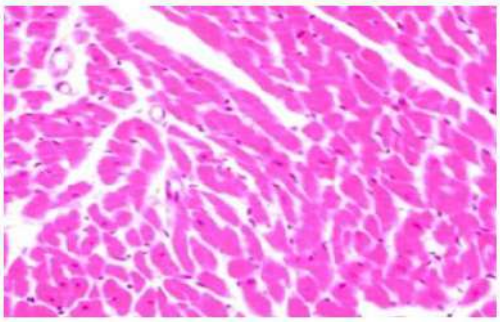
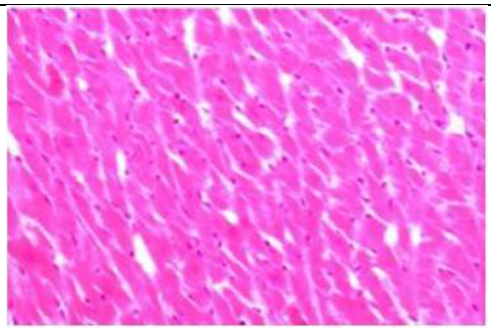
|  |  |
| **Group 1: Vehicle Control rats showing Normal histoarchitecture of Heart tissue** | **Group 2: Cyclophosphamide induced Cardiotoxiocity rat model (150 mg/kg, b.wt.i.p.) Sections of heart ventricle from the group that received cyclophosphamide showed injured myocytes with scattered coagulative changes and thin bands of contraction necrosis.** |
|  |  |
| **Group 3: Cyclophosphamide + HAHU-I (Low Dose extract) Sections of heart from the low dose test showed individual cardiac muscle cells arranged in diffuse bundles in a connective tissue framework. Individual myocytes are seen in cross section to be well stained and preserved.** | **Group 4: Cyclophosphamide + HAHU-II (High Dose extract) Sections of heart from the high dose test showed centrally located nuclei with abundant cytoplasm outlined by distinct and intact cell walls. The myocytes essentially appear normal.** |

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**RESEARCH ARTICLE**

# Parkinson's Disease Detection using Machine Learning Approach

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**ABSTRACT**

One of the very common neurological disorders is Parkinson's disease (PD) where movements of the muscles are restricted in the body. It has a major impact on posture, speech, and mobility; symptoms include tremors, muscle rigidity, and slower movement. The disease is caused by a decrease in dopamine levels in the brain as a result of the loss of neurons. Motor performance is hampered by low dopamine levels because they interfere with nerve cell-to-nerve cell signal transmission. Balance issues and tremors are common side effects of dopaminergic neuron degeneration, while individual differences may exist in the course and intensity of symptoms. Parkinson's disease (PD) currently has no known cure; As such early detection of this disease is very important. In this article we have tried to identify different symptoms of this disease. We have tried to collect data symbols and classified them using different machine leaning algorithms.

**Keywords**: Parkinson's disease, artificial intelligence, diagnosis, data integration, machine learning, deep learning, voice signal analysis, Neurodegenerative disorders.

## INTRODUCTION

One of the very common neurological disorders is Parkinson's disease (PD) where movements of the muscles are restricted in the body. It has a major impact on posture, speech, and mobility, which results different symptoms like the rigidity of the muscle and other problems [1]. Because of the reduction of the dopamine levels in the human brain

this disease is created. Motor performance is hampered by low dopamine levels because they interfere with nerve cell-to-nerve cell signal transmission. The symptoms of dopaminergic neuron degeneration can vary in severity from person to person, and they can include tremors and balance issues. As of now, there exists no cure for Parkinson's disease. As such an early detection of the same is very much important. Sensor based remote health monitoring is a very common and an important topic for research. A lot of research work is going on this throughout the globe [8-18]. In this research we have used the concept of remote sensor based health monitoring system.

There are five different stages of Parkinson's disease (PD), and 90% of PWP have vocal cord damage, which is frequently noticeable as a symptom at stage 0. In addition to providing a quantifiable measure, vocal abnormalities are also covered by telemedicine [2] or remote medical care. Patients can record short audio clips at home using their phones. Dysphonia and dysarthria are common symptoms associated with vocal modulation [3].Preliminary results show that the Random Forest classifier model outperforms KNN, SVM, and Logistic Regression models with an accuracy rate of more than 91% when trained on attributes taken from MDVP audio data. In section II a brief literature survey has been given, research gap has been identified in section III, problem statement has been described in section IV, experimental data collected in section V, result analysis has been shown at section VI and section VI concludes the paper.

## LITERATURE SURVEY

Previous research on the use of MRI scans, genetic data, and gait analysis to predict Parkinson's disease (PD) has been conducted; however, little is known about the role that hearing impairment plays in early identification. For example, Bilal et al. used an SVM model to predict the onset of Parkinson's disease (PD) in older patients based on genetic data. In contrast to the current study, which describes an improved SVM model with an accuracy of 0.9183, their SVM model attained an accuracy of 0.889. These results highlight the benefits of using auditory data for PD classification rather than genetic data. Using keystroke data from the UCI telemonitoring dataset, Raundale, Thosar, and Rane [4] trained a Random Forest classifier to predict the severity of Parkinson's disease in elderly people. For PWP classification, Cordella et al. used audio data; MATLAB was used in their models. Our study, however, makes use of models trained in Python that are publicly available.

Most earlier studies have emphasized the use of deep learning for Parkinson's disease (PD) identification. For instance, Ali et al. clarified how to estimate Parkinson's disease development using ensemble deep learning models applied to phonation data. Their research, however, lacked feature selection methods to improve the performance of Deep Neural Networks. In order to identify 7 primary voice modes in PD identification, the paper uses primary Component Analysis (PCA) on 22 variables. In order to reduce the reliance of Parkinson's disease (PD) diagnosis on wearable technology, Huang et al. [5] trained a traditional decision tree using 12 sophisticated speech features from the MDVR-KCL dataset. Instead of concentrating on the subtleties of audio frequency, Wodzinski et al. trained a ResNet model on pictures of audio data. Wroge et al.'s [6] use of an unbiased machine learning model aimed to reduce the subjectivity of doctors in PD prognosis.

**Identification of the research gap**

Significant gaps in early research persist because of inadequate examination of the integration of various profiles, despite advancements in the use of AI and ML for the early identification of Parkinson's disease (PD). Earlier studies focused on individual data types. The benefits of combining motor symptoms, demographics, genetics, and neuroimaging are unexplored. This integration could enhance understanding of early disease manifestations. Many studies use controlled datasets that don't represent real-world complexity. Including data from longitudinal studies and diverse demographics is essential to improve the generalizability of AI models for early PD detection, ensuring reliability across diverse patients. Early studies overlooked individual differences. Customizing AI models for unique patient characteristics is crucial. Advances in AI promise more accurate, personalized, and timely Parkinson's diagnosis, improving outcomes.

**Problem Statement**

This research intends to advocate for the integration of machine learning techniques in telemedicine. By doing so, it aims to offer renewed hope and improved life quality to individuals grappling with Parkinson's disease. This research aims to develop an AI-based system for PD diagnosis, integrating multiple data sources to improve on current subjective and error-prone methods. The study explores ML in telemedicine for early PD detection, training four models on audio data from 30 patients and healthy individuals. The Random Forest model proved most effective, achieving 91.83% accuracy and 0.95 sensitivity for PD detection. In fig. 1 a workflow diagram of the system is drawn.

**Experimental data collection**

For our experiment necessary data has been collected in cooperation with the National Centre for Voice and Speech in Denver, Colorado, Max Little of the University of Oxford generated the dataset. In this process voice samples and other features has been extracted from different people some of whom suffer from Parkinson's disease. Number of instances collected are 195. Attributes number are 24. They are of real value type. And the target variable status has been recorded as 1 for the Parkinson's disease people and 0 for the healthy people. Data has been read from the panda data frame for easy manipulation and analysis (fig. 2 and fig. 3). Analysis is done based on different models like

**Model Selection**

Various machine learning algorithms are chosen to compare their performance. Machine learning models include Logistic Regression, Decision Tree, Random Forest, Support Vector Machine (SVM), K-Nearest Neighbors (KNN), Gaussian Naive Bayes, Bernoulli Naive Bayes, Voting Classifier, XGBoost.

Fig. 5: Data model

**RESULT ANALYSIS**

Results of different machine learning models have been implemented and the result is given below:

## CONCLUSION

It has shown encouraging results to classify Parkinson's disease (PD) based on vowel phonation data. Because it took into account all 22 features in the MDVP dataset, the Random Forest classifier was able to attain above 98% accuracy in classifying Parkinson's disease using vowel phonation data. The accuracy and sensitivity of the SVM model were 0.94 and 91.836%, respectively, after PCA was administered. The K Nearest Neighbours (KNN) model benefited from its capacity to classify data without prior assumptions, showing the highest precision and recall values, both at 0.95, on a balanced dataset. Based on voice features, the Voting Classifier and Random Forest models distinguished between people with Parkinson's disease and those who were healthy with a robust accuracy of 92.3%. The models' performance was greatly enhanced by the usage of Random over Sampler to handle class imbalance and PCA for dimensionality reduction. Even with these encouraging outcomes, hyper-parameter tweaking and investigating sophisticated ensemble methods could yet be improved. Because the Random Forest model is simple, accurate, and non-invasive, we advise using it to classify illness progression. Still, auditory data alone might not be sufficient for accurate classification. Thus, through telemedicine, combining audio data with REM sleep data could improve classification accuracy.

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**Table 1. Results of different machine learning models have been implemented and the result is given below:**

| | |
|---|---|
| Logistic Regression | Used Logistic Regression with parameters C=0.4 and max_iter=1000. |
| Decision Tree Classifier | Used Decision Tree Classifier with a random state for reproducibility. |
| Random Forest Classifier | Implemented two versions with different criteria: gini and entropy. |
| Support Vector Classifier (SVC) | Implemented using SVC with default parameters. |
| K-Nearest Neighbors (KNN) | Implemented using KNeighbors Classifier with default parameters. |
| Naive Bayes Classifiers | Implemented Gaussian NB and Bernoulli NB. |
| Voting Classifier | Combined all above models using Voting Classifier with hard voting. |
| XGBoost Classifier | Implemented using XGB Classifier with default parameters. Evaluated using accuracy score. |

**Table 2: Different models and their values.**

| | |
|---|---|
| Logistic Regression | 0.779661 |
| Decision Tree | 0.98351 |
| Random Forest (Gini) | 1.000000 |
| Random Forest (Entropy) | 1.000000 |
| SVC | 0.949153 |
| KNN | 0.949153 |
| GaussianNB | 0.762721 |
| BernoulliNB | 0.745763 |
| Voting Classifier | 0.966102 |

**Table 3.Evaluated the performance using confusion matrix and classification report for models with high accuracy which is described below.**

| Training Confusion Matrix: | Testing Confusion Matrix: |
|---|---|
| [[81 0]<br>[ 0 75]] | [[30 2]<br>[ 2 5]] |
| Training Classification Report:<br>precision recall f1-score support<br>0 1.00 1.00 1.00 81<br>1 1.00 1.00 1.00 75<br>accuracy 1.00 156<br>macro avg 1.00 1.00 1.00 156<br>weighted avg 1.00 1.00 1.00 156 | Testing Classification Report:<br>precision recall f1-score support<br>0 0.94 0.94 0.94 32<br>1 0.71 0.71 0.71 7<br>accuracy 0.90 39<br>macro avg 0.82 0.82 0.82 39<br>weighted avg 0.90 0.90 0.90 39 |

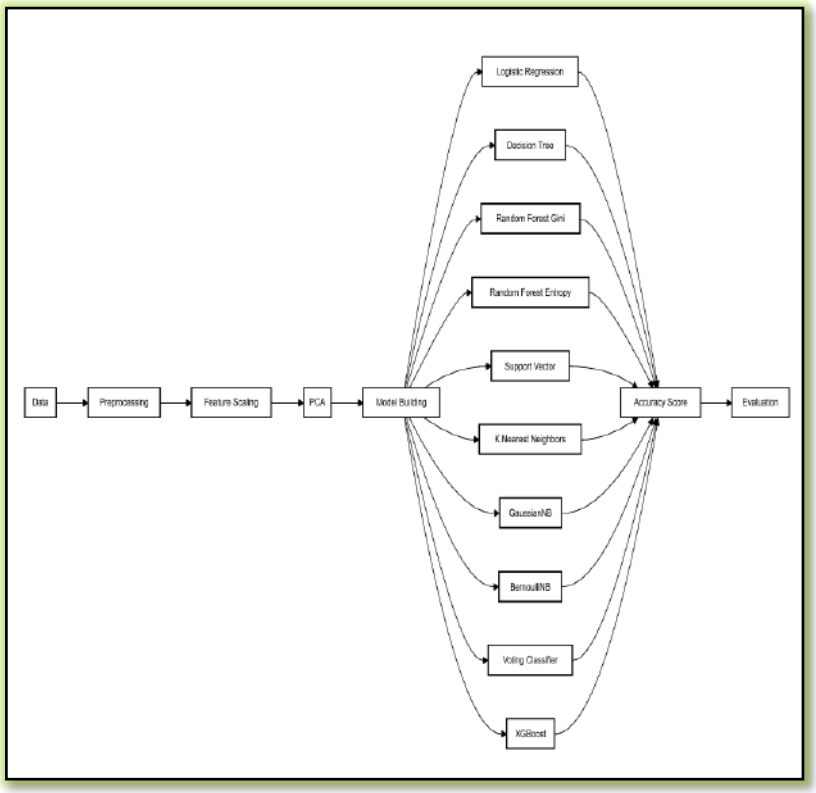



**Fig.1: Workflow Diagram**

| | name | MDVP:Fo(Hz) | MDVP:Fhi(Hz) | MDVP:Flo(Hz) | MDVP:Jitter(%) | MDVP:Jitter(Abs) | MDVP:RAP | MDVP:PPQ | Jitter:DDP | MDVP:Shimmer |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | phon_R01_S01_1 | 119.992 | 157.302 | 74.997 | 0.00784 | 0.000070 | 0.00370 | 0.00554 | 0.01109 | 0.04374 |
| 1 | phon_R01_S01_2 | 122.400 | 148.650 | 113.819 | 0.00968 | 0.000080 | 0.00465 | 0.00696 | 0.01394 | 0.06134 |
| 2 | phon_R01_S01_3 | 116.682 | 131.111 | 111.555 | 0.01050 | 0.000090 | 0.00544 | 0.00781 | 0.01633 | 0.05233 |
| 3 | phon_R01_S01_4 | 116.676 | 137.871 | 111.366 | 0.00997 | 0.000090 | 0.00502 | 0.00698 | 0.01505 | 0.05492 |
| 4 | phon_R01_S01_5 | 116.014 | 141.781 | 110.655 | 0.01284 | 0.000110 | 0.00655 | 0.00908 | 0.01966 | 0.06425 |
| 5 | phon_R01_S01_6 | 120.552 | 131.162 | 113.787 | 0.00968 | 0.000080 | 0.00463 | 0.00750 | 0.01388 | 0.04701 |
| 6 | phon_R01_S02_1 | 120.267 | 137.244 | 114.820 | 0.00333 | 0.000030 | 0.00155 | 0.00202 | 0.00466 | 0.01608 |
| 7 | phon_R01_S02_2 | 107.332 | 113.840 | 104.315 | 0.00290 | 0.000030 | 0.00144 | 0.00182 | 0.00431 | 0.01567 |
| 8 | phon_R01_S02_3 | 95.730 | 132.068 | 91.754 | 0.00551 | 0.000060 | 0.00293 | 0.00332 | 0.00880 | 0.02093 |
| 9 | phon_R01_S02_4 | 95.056 | 120.103 | 91.226 | 0.00532 | 0.000060 | 0.00268 | 0.00332 | 0.00803 | 0.02838 |
| 10 | phon_R01_S02_5 | 88.333 | 112.240 | 84.072 | 0.00505 | 0.000060 | 0.00254 | 0.00330 | 0.00763 | 0.02143 |
| 11 | phon_R01_S02_6 | 91.904 | 115.871 | 86.292 | 0.00540 | 0.000060 | 0.00281 | 0.00336 | 0.00844 | 0.02752 |
| 12 | phon_R01_S04_1 | 136.926 | 159.866 | 131.276 | 0.00293 | 0.000020 | 0.00118 | 0.00153 | 0.00355 | 0.01259 |
| 13 | phon_R01_S04_2 | 139.173 | 179.139 | 76.556 | 0.00390 | 0.000030 | 0.00165 | 0.00208 | 0.00496 | 0.01642 |
| 14 | phon_R01_S04_3 | 152.845 | 163.305 | 75.836 | 0.00294 | 0.000020 | 0.00121 | 0.00149 | 0.00364 | 0.01828 |

✓ 0s completed at 3:38 AM

**Fig. 2: Dataset for analysis**

| | MDVP:Fo(Hz) | MDVP:Fhi(Hz) | MDVP:Flo(Hz) | MDVP:Jitter(%) | MDVP:Jitter(Abs) | MDVP:RAP | MDVP:PPQ | Jitter:DDP | MDVP:Shimmer | MDVP:Shimmer(d |
|---|---|---|---|---|---|---|---|---|---|---|
| count | 195.000000 | 195.000000 | 195.000000 | 195.000000 | 195.000000 | 195.000000 | 195.000000 | 195.000000 | 195.000000 | 195.0000 |
| mean | 154.228641 | 197.104918 | 116.324631 | 0.006220 | 0.000044 | 0.003306 | 0.003446 | 0.009920 | 0.029709 | 0.2822 |
| std | 41.390065 | 91.491548 | 43.521413 | 0.004848 | 0.000035 | 0.002968 | 0.002759 | 0.008903 | 0.018857 | 0.1948 |
| min | 88.333000 | 102.145000 | 65.476000 | 0.001680 | 0.000007 | 0.000680 | 0.000920 | 0.002040 | 0.009540 | 0.0850 |
| 25% | 117.572000 | 134.862500 | 84.291000 | 0.003460 | 0.000020 | 0.001660 | 0.001860 | 0.004985 | 0.016505 | 0.1485 |
| 50% | 148.790000 | 175.829000 | 104.315000 | 0.004940 | 0.000030 | 0.002500 | 0.002690 | 0.007490 | 0.022970 | 0.2210 |
| 75% | 182.769000 | 224.205500 | 140.018500 | 0.007365 | 0.000060 | 0.003835 | 0.003955 | 0.011505 | 0.037885 | 0.3500 |
| max | 260.105000 | 592.030000 | 239.170000 | 0.033160 | 0.000260 | 0.021440 | 0.019580 | 0.064330 | 0.119080 | 1.3020 |

**Fig. 3: Dataset 2 for analysis**

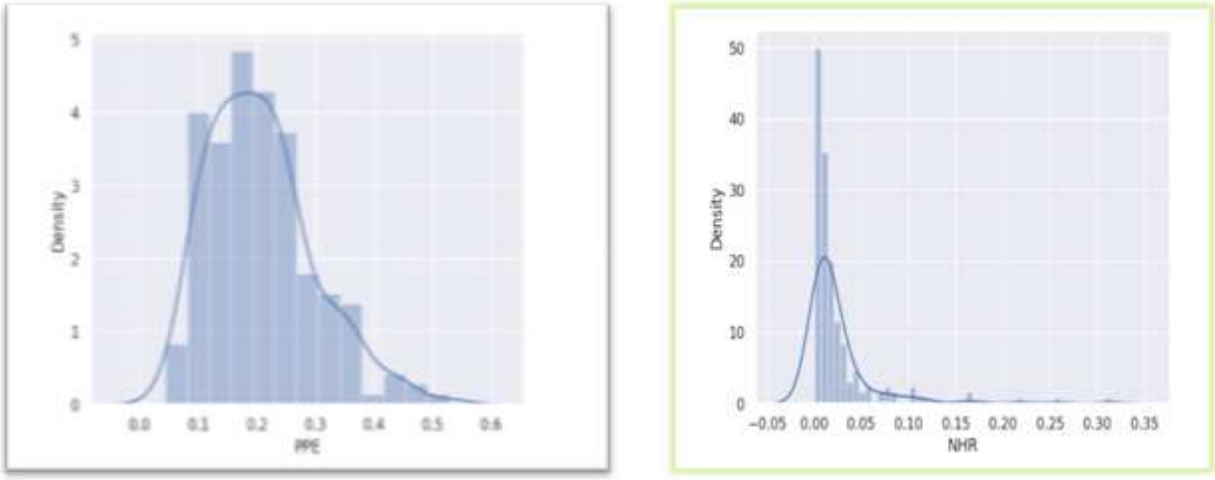



**Fig. 4: Distribution of each feature using Seaborn's distplot.**

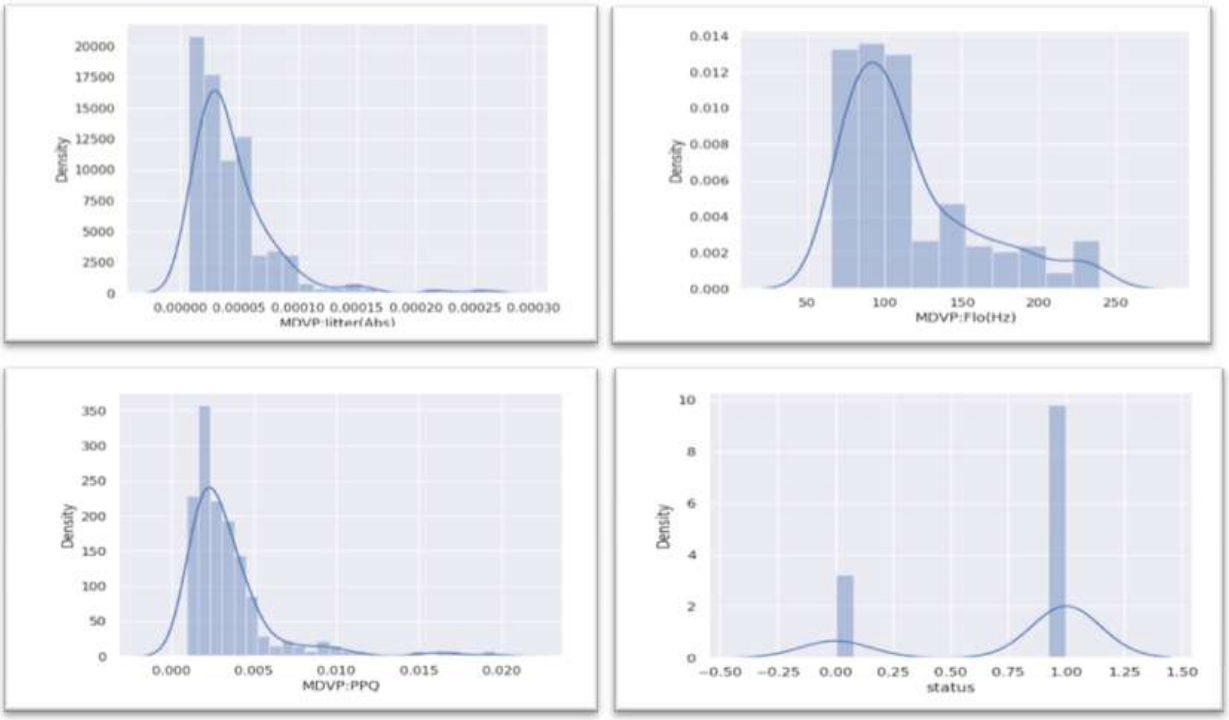
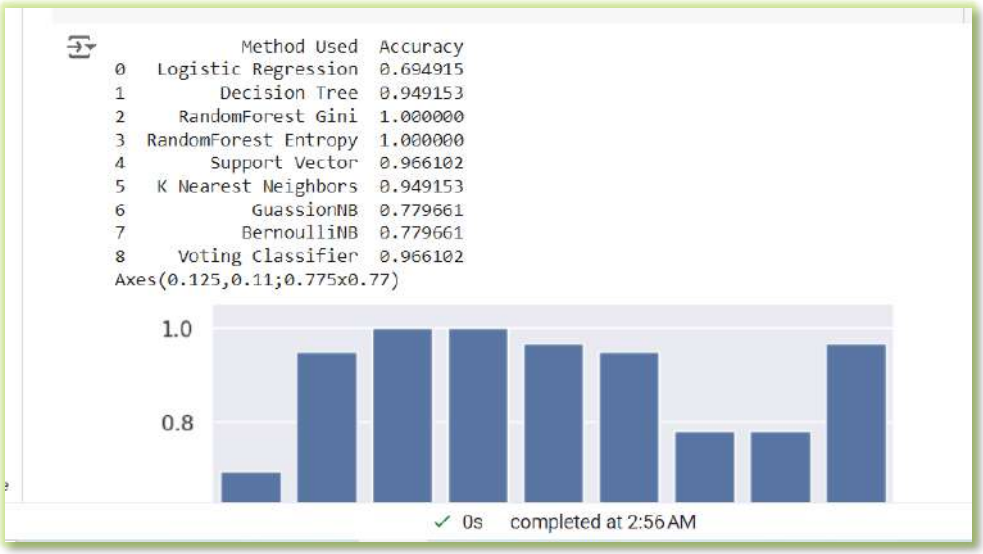



**Fig. 5: Data model**



```
            Method Used  Accuracy
0   Logistic Regression  0.694915
1         Decision Tree  0.949153
2     RandomForest Gini  1.000000
3  RandomForest Entropy  1.000000
4        Support Vector  0.966102
5   K Nearest Neighbors  0.949153
6            GuassionNB  0.779661
7           BernoulliNB  0.779661
8     Voting Classifier  0.966102
Axes(0.125,0.11;0.775x0.77)
```



**Fig.6. Different models and their values**

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**RESEARCH ARTICLE**

# Effect of Inadequate Sleep on Memory and Learning: a study on Adolescents

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**ABSTRACT**

The role of sleep is very important for several important cognitive processes. In developing adolescents, getting adequate sleep is even more important as it affects the development of intellectual ability, learning and memory, reward and motivation, emotional regulation, etc. Adequate sleep for adolescents varies from 7-10 hours. But a majority of adolescents get less than 7 hours of sleep daily. This review discusses the effect of this inadequate sleep on memory and consequently its effect on learning and academics. The results indicate that due to a variety of reasons, adolescents ended up getting as little as 5-6 hours of sleep on weeknights. Thus their short-term memory was affected negatively and they did not learn as well as their peers who slept well.

**Keywords:** Inadequate sleep, memory, learning, adolescents

## INTRODUCTION

Sleep can be defined as a state of reduced mental and physical activity that typically recurs for several hours every night where the consciousness of the surroundings is practically suspended and sensory activity is inhibited partially. (Chokroverty S, *et al*, 2010). A lot of studies have linked the role of sleep to cognitive processes like alertness, perception, emotion, learning and memory, and executive functioning. (Killgore, *et al*, 2014). Now the three factors that affect cognitive performance and higher-order functions are inertia, homeostasis, and circadian rhythms. (Kapsi, S., *et al*, 2020). Burke *et al*. claim that homeostasis and circadian cycles are the two factors controlling working memory, mood, alertness, motivation, and visual attention maintenance. (Burke, T. M., 2015) This means that getting

less sleep may result in a decrease in these cognitive functions. Studies also suggest that, with inadequate sleep, behavior becomes increasingly irregular and unstable, attentional lapses become longer and more frequent, (Lim J, 2010) and simple reaction time is slowed (Lim J, 2008). Since memory and learning are some of the most important cognitive skills required for students, this review paper mainly deals with establishing the negative effects of inadequate sleep on them. The sample population taken into account here is adolescents. According to the definition given by the World Health Organisation, children from 10-19 years are considered adolescents. A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society states that adolescents require about 7-10 hours of sleep (Consensus Conference Panel, 2015) (Chaput, J. P. 2018).

**Sleep Patterns in Adolescents**

Sleep patterns refer to the regular and recurring cycles of sleep stages that individuals go through during a typical night of sleep. These patterns are characterized by different phases, including both rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. Now, sleep patterns in adolescents are mostly predictable. Their nocturnal sleep schedules decrease and bed-times are delayed. (Gariepy, G., *et al* 2020). But school/college timings do not change and thus adolescents get less sleep than recommended. Hysing M, *et al*, report from their study conducted in Hordaland County in Norway, consisting of 10,220 adolescents aged 16–18 years (54% girls), that there was a sleep deficit of about 2 hours on weekdays and latency of sleep onset exceeding 30 min on 65% of those studied. Insomnia rates were higher in girls whereas boys reported later bedtimes. (Hysing M, *et al*, 2013). Bed times tend to delay with increasing age according to meta-analysis of worldwide adolescent sleep patterns in the last decade (1999–2010). Asian adolescents slept even less than peers from North America and Europe. This resulted in less total sleep duration on school nights and a tendency for greater degrees of daytime drowsiness. (Gradisar, M, 2011). Another study corroborating this was conducted in Delhi, India among 501 students (aged 11–15 years). This study provides statistical proof of sleep deprivation increasing with age. Sleep deprivation increased 83.7% to 87.1% in 11–12 y to 90.5% to 92.5% in 13–15 y. (Singh, R., 2018). Only 33% college students in America reported sleeping a full 8 hours/night. Sleep interruptions were caused by social media usage, gaming, and texting. (Whipps, J, 2018). Students aged 17-24 at a large private university in the Mideast reported chronically restricted sleep. 25% of students got less than 6.5 hours/night and only 29.4% of students reported getting 8 hours of sleep which is the typical hours required for adolescents. (Lund, H. G., *et al*, 2010). According to the Youth Risk Behavior Survey, 72.7% of students said they slept for less than eight hours on school nights on average. (A. G. Wheaton *et al*., 2018). This is comparable to the National Sleep Foundation poll, which found that 62% of students slept for less than eight hours on weeknights. With 75% of seniors reporting less than 8 hours of sleep every night, they were the group most sleep deprived. (Carskadon, M. A., *et al* 2015) Girls were the ones disproportionately affected. (Eaton, D. K., *et al* 2010). We can thus infer that most adolescents worldwide sleep less than required. Older adolescents also slept less than their younger counterparts. (Gariepy, G., *et al* 2020).

**Factors Affecting Sleep In Adolescents**

Adolescents are notorious for getting inadequate sleep. There may be some reasons for that. It could be academic pressure, developmental changes during puberty, social media usage, gaming, peer pressure, parental pressure, increased usage of caffeine or other energy beverages, decreased curfew and increased bedtimes, and certain mental problems. Puberty is the time when humans undergo a lot of changes. One of them is the shift of the timing of circadian rhythm. Before puberty, the body shows signs of sleep around 8:00 or 9:00 pm. This cycle changes when puberty sets in and the body only feels sleepy at around 10:00 or 11:00p.m. (Burke, T. M., *et al* 2015) "Sleep phase delay" refers to the normal circadian rhythm shift experienced by teenagers. The need to sleep is delayed by around two hours. Therefore adolescents struggle to go to sleep at their regular hour. But they still require nine hours of sleep every night on average which is impossible with early school/college start times. (Gariepy, G., *et al* 2020) This is where adolescents struggle with getting adequate sleep. Lohsoonthorn, V *et al* report another contributing factor. 48.1 % of Thai college students reported poor sleep quality. 58% of the same subjects were using some sort of stimulant beverage. Hence stimulant use, alcohol consumption and cigarette smoking were positively associated with poor sleep quality and increased daytime sleepiness. (Lohsoonthorn, V *et al* 2013). Sanchez, S. E., *et al* conducted a similar study among 2,458 Peruvian college students. Those who reported consuming more than 3 stimulant beverages per

week had higher odds of poor sleep quality, short sleep duration and increased use of sleep medication. (Sanchez, S. E., *et al* 2013) Whereas another study comprising 11025 students aged 17 to 24 years from an urban Midwestern university found that alcohol and caffeine consumption, exercise, and consistency of sleep schedule were not significant contributors to sleep quality in the 60% of students who were reported as poor sleepers. Instead, emotional and academic stress was the cause affecting sleep. (Lund, H. G., *et al* 2010) When a total of 937 ninth-grade adolescents (15–16 years), from western Sweden participated in a study, 55% of the adolescents were found to sleep less than the recommended 8 hours per night due to school stress and technology use. 11% also had sleeping difficulties. (Jakobsson, M., *et al*, 2019) Students experience high stress during the school year. The majority of them also report getting only 6 hours of sleep during weekdays. This leads to them experiencing moderate fatigue and more depressive symptoms. High-stress levels are associated with sleep disturbances, less nocturnal total sleep time, and higher fatigue severity. (Lee, S. Y., *et al*, 2013) It was found that sleep was more fragmented during high-stress times in an experiment comparing the quality and duration of sleep in adolescents during low and high-stress times. (Dewald, J. F., *et al*, 2014) Whipps, J., *et al* reported in a study evaluating night time media use and sleep patterns in college students, that students had a mean sleep duration of only 7.26 ± 0.93 hours. Reports of texting after bed and device-related sleep interruptions were many. Social media usage, mobile gaming, and texting were correlated with sleep interruptions. (Whipps, J., *et al*, 2018) Young adults (aged 19-32) in the US reported an average of 61 min of social media usage per day. More than half of the same group reported medium or high levels of sleep disturbance. The results did not vary even when social media usage volume or frequency was taken as the variable. (Levenson, J. C., *et al* 2016). Wong, H. Y., *et al* assessed Hong Kong university male students in 2019 on the relationship between the severity of internet gaming disorder and poor sleep quality and found a direct relationship between both. (Wong, H. Y., *et al* 2020). Another similar study conducted among 524 adolescents in Lebanon found that those with Internet Gaming Disorder slept only 5 hours per night as opposed to those who casually played online games and slept for 7 hours. (Hawi, N. S., *et al*, 2018) To find if there was a relationship between obesity and sleep, 515 college students were assessed. It was found that one-third of the participants had with BMI of more than 25 and 51% of those had poor-quality sleep. Sleep disturbances were thus a result of obesity and sleep duration was not affected. (Vargas, P. A., *et al* 2014) When a study on the impact of sleep disorders was conducted in the US for 6139 individuals over the age of 16, it was found that 4.2% had sleep apnea, followed by 1.2% having insomnia and RLS (0.4%). Common sleep habits were snoring (48%), feeling unrested during the day (26.5%), and not getting enough sleep (26%). It was also found that insomnia, sleep apnea, and RLS had the highest impact on concentration and memory. (Ram, S., *et al*, 2010) Dyssomnias, especially early and middle insomnias (odds ratio = 1.74 and 2.24), disturbed the circadian rhythm by prolonging bedtimesin a study conducted with 1253 adolescents in grades 3, 5, and 8. As a consequence duration of sleep also decreased. (Chen, Y. L., 2016). Another factor responsible for inadequate sleep in adolescents was outlined by Crowley SJ *et al*, in 2010. According to them, during the second decade of life sleep/wake timing shifts to later, and Delayed Sleep Phase Syndrome (DSPS), may be an extreme manifestation of this. (Crowley SJ *et al*, 2010) Thus some factors affecting sleep in adolescents and causing their sleep durations to decrease were academic and emotional stress, consumption of a lot of caffeinated and energy drinks, usage of social media, severe internet gaming disorders, dyssomnias, and delaying of sleep/wake timing. A factor that did not contribute to disturbing sleep duration was obesity.

**Link between Sleep and Memory**

Memory is the capacity to store and retrieve information. (Zlotnik, G., & Vansintjan, A. 2019) Squire, L. R defines memory as 'the faculty of encoding, storing, and retrieving information'. Psychologists have found that memory includes four important categories: working, sensory, short-term, and long-term. According to the definition of the American psychological association, sensory memory holds sensory information for very less time, usually 1 second or less. (VandenBos, G. R. 2007). Short-term memory allows a person to recall limited information for a short period of time. (Cascella, M., & Al Khalili, Y. 2019). Working memory is similar to short-term memory but here the person manipulates information. Long-term memory stores a wide array of memories and experiences. The majority of the memories that people can recall, especially those that are more than thirty seconds old are part of long-term memory. Long-term memories are divided into two subcategories: implicit and explicit. Explicit memories are conscious experiences of events, personal anecdotes, or lessons learnt. Some types of explicit long-term memory include

episodic memory and semantic memory. Implicit memories are memories that influence a person's behaviour unconsciously. Some types of this memory include procedural memory and priming/conditioning. (Cowan, N. 2008). Zhang's memory model explains the importance of sleep in memory. This model has 2 stages: waking and sleeping brain. During waking time, the memories created in our working memory during the waking state are stored in temporary memory. Because there is a limited amount of temporary memory, it has to be periodically cleansed to avoid overload. Thus during sleeping time, the temporary data is supposed to be compared with old data saved in the long-term memory, in order to identify and delete unwanted, duplicate and overlapping data. The remaining information is then encoded and transferred to long-term memory. When the working memory is overloaded with incoming data, our waking brain is unable to complete this task. The temporary memory must be isolated from the outside world in order to carry out this housekeeping and guarantee that the memory transfer procedure continues unhindered. This isolation is carried out while sleeping. Sleep has the purpose of processing, encoding, and transferring information from the temporary memory to the long-term memory according to this memory model. (Zhang, J. 2004) This model is the updated version of the memory consolidation model proposed by Squire, L. R., & Alvarez, P in 1995.

Experimentally, the role of sleep in explicit memory was proved by measuring the memory retention of 29 adolescents (aged 15–18 years) who were given only 5 hours of sleep opportunity each night. Retention was tested 30 minutes, 3 days and 42 days (only a subset with n=14 participated) after learning and results were compared to a control group (n = 30) that slept for nine hours every night during the trial. 26% forgot at the 30-minute test, 34% at the Day 3 test, and 65% at the Day 42 test. (Cousins, J. N., *et al* 2019). Sleep facilitates the consolidation of declarative, procedural, and emotional memories. (Diekelmann, S., & Born, J. 2010) Emotional memory was found to be consolidated more during REM sleep participants reporting depressive symptoms. Additionally, those depressive participants who were sleep deprived consolidated significantly less emotional (negative here) information. (Harrington, M. O., at al 2018). Sleep helps in working memory as well as memory consolidation in children and adolescents. It was further found that sleep deprivation negatively affected complex tasks involving higher brain functions more strongly than performance in simple memory tasks. (Kopasz, M., *et al* 2010) Research reveals a decrease in working memory, sustained attention, and executive functions in sleep restricted (SR) groups, while the control group maintains baseline levels. It was found that the SR group continued to perform worse than the control even after 2 recovery sleep episodes. (Lo, J. C., 2016). A review study conducted by Wilhem L., *et al* supports this and further states that slow-wave sleep plays a major role in the consolidation of procedural memory. (Wilhelm, I., 2012) Further evidence comes from a study by Fenn, K. M., &Hambrick, D. Z. When memory was tested after a delay of roughly 12 hours that included sleep or waking, it was found that those who slept remembered more and forgot less than those who were awake. They hypothesize that sleep enhances the retrieval of information and also protects against loss of memory that occurs when awake.(Fenn, K. M., &Hambrick, D. Z.2013) A study conducted by Ashton, J. E., *et al* on sleep deprivation found that rates of forgetting in episodic memory increase with sleep deprivation. Overnight sleep deprivation was also found to lead to further deficits in associative memory that were not observed after daytime wakefulness. (Ashton, J. E., *et al*, 2020) Partial sleep restriction had small or no effects on adolescent cognitive functioning. However, where sleep deprivation was observed, there was a decrease in psychomotor tasks. (Whitney P., *et al*, 2010). It was also found that sleep extension and sleep improvement caused improvement in working memory. When subjects slept directly after learning, they also improved memory consolidation. (De Bruin, E. J., *et al* 2011) Looking at the evidence provided by the studies stated above and a lot of other growing evidence, we can safely conclude that sleep is important for all types of memory consolidation.

**Relation between Sleep, Memory and Learning**

There are 2 types of memory. They are short and long term memories. The primary function of short term memory/working memory is to support learning. (Gathercole, S. E. 2004, January). Therefore for adolescents whose main duty is to learn, usage of this cognitive skill is very important. The type of memory most important while learning is working memory as it refers to a brain system that allows temporary storage and manipulation of the information necessary for complex cognitive. (Baddeley, A. 1992). After conducting a thorough meta-analysis of the literature on these characteristics in kids, Astill *et al*. found that sleep duration was positively and significantly

correlated with executive functioning, academic achievement, and multiple-domain cognitive functioning. (Astill *et al*., 2012) It was also found that children who slept earlier did better on cognitive tests. (Fonseca, A. G., 2020). Research indicates that while we sleep, the brain performs neurological processes related to learning and memory, processing information that is gained while we are awake (Watson and Buzsáki, 2015). Sleep has been shown to improve performance on several learning and memory tasks, such as the face sequence recognition task, the motor adaptation task, the word-pair association task, the visual texture discrimination task, and the motor sequence task.( Uji, M., & Tamaki, M. 2023) When 7798 adolescents aged 16–19 years were surveyed to link sleep and academic performance in Norway, it was found that students with sleep deficits and short sleep duration had the highest odds of poor GPA. ( Hysing, M., *et al* 2016) In tests where researchers were able to deliberately alter sleep to track behavioral and neurocognitive outcomes; including learning, memory, and academic achievement, it was primarily found that both the quantity and quality of sleep have a direct impact on students' academic performance and learning capacity. Sleep loss was also frequently linked to students' poor declarative and procedural learning. Further, there was a decline in neurocognitive and academic performance. (Curcio, G., *et al*, 2016) All things considered, behavioural research on adolescents suggests that sleep is essential for memory consolidation. All stages of sleep have been linked to one or more components of this consolidation up until this point of sleep. (Walker, M. P., &Stickgold, R. 2004). However, a comprehensive knowledge of the functions of each stage of sleep is still a crucial future objective. It was found that students who performed the best academically had far earlier waking times ($p = 0.008$) and bedtimes ($p = 0.05$) than those who performed the worst. High achievers tended to nap more frequently ($p = 0.07$). (Eliasson, A.H.,2010) Even when alertness and vigilance are restored with stimulant countermeasures, some aspects of higher-level cognitive capacities are still impaired by sleep deprivation, indicating that sleep loss may have effects on particular cognitive systems in addition to those caused by attentional processing impairments or general cognitive declines. (Killgore, W. D. 2010) This was further proved in a study conducted among 1,845 college students at a large, southeastern public university, where it was found that students reported insufficient sleep and those same students were also at the risk of academic jeopardy with GPAs of less than 2. (Gaultney, J. F. 2010).

## DISCUSSION AND CONCLUSION

A lot of significant research shows that a majority of adolescents are getting inadequate sleep mostly on weekdays (Hysing M, *et al*, 2013) and it is risking their memory and learning processes thus affecting their academic performance. It is thus imperative that measures be taken to make sure that adolescents get enough sleep. Some solutions for the above may be later school/college timings (de Araújo, L. B. G., *et al* 2020), earlier curfews and bedtimes (Campbell, I. G., *et al* 2023), implementation of naps in between learning (Inazumi, C. K., *et al* 2020), avoidance of social media (Levenson, J. C., *et al* 2017) and early diagnosis and cure for sleep disorders. (Modlin, T. 2002). It is thus established that there is a significant link between inadequate sleep, memory, and learning in adolescents. Inadequate sleep decreases both memorizing ability and thus learning ability is also decreased. It is also linked to low performance in academics.

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**RESEARCH ARTICLE**

# Mathematical Model with an Application of New Topsis Model Approach to Identify the Over Spread of Typhoid

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**ABSTRACT**

A pandemic is an illness that spreads rapidly over a huge region, such as several continents or the entire world. A potentially fatal infection, typhoid fever is brought on by the salmonella Typhi bacteria. Typhoid fever is thought to infect 11–20 million people annually, killing between 1,28,000 and 1,61,000 of them. Finding the important risk factor is the primary goal of the current inquiry. This study uses the Topsis MCDM technique to evaluate and identify the spread of typhoid.

**Keywords:** Topsis MCDM, Fuzzy Topsis MCDM, Topsis Multi Criteria Decision, MCDM tools.

## INTRODUCTION

A severe, contagious, and potentially fatal illness associated with fever is typhoid. Salmonella enterica serovars are to blame. While some are grouped as nontyphoidal Salmonella [NTS] [1], Typhi, Paratyphi A, B, and C can all be classified as typhoidal Salmonella. Enteric fever is the collective term for typhoid and paratyphoid fever, which are caused by human host-confined typhoid strains. Salmonella serovar Paratyphi A has been associated with an increasing incidence of enteric fever in certain Asian countries [2, 3]. The primary means of transmission for typhoidal Salmonella is contaminated water or food [4]. In developing countries where typhoidal Salmonella is widespread, there is inadequate sanitation and hygiene, and there is a shortage of safe water and food, there is a

greater risk of illness [5]. An ongoing study on the prevalence of typhoid fever worldwide found that the disease causes 27 million illnesses and 200,000–600,000 deaths annually [7]. In 2010 there were 11.9 million cases of typhoid fever and 129,000 deaths in low- and middle-income countries, according to the International Vaccine Institute [8]. For the most part, typhoid is endemic in developing countries like Pakistan [9].

We attempt to search for any potential escape route as the virus spreads over national borders. Medical science is making every effort to find a potential remedy. Even in the non-medical field, we have made an effort to contribute in some small way to the cause. Based on information gathered thus far from literature surveys, media websites, and medical advice, we have identified three primary causes of typhoid disease transmission. The main ways that it spreads are through contaminated food or water, through contact with an infected person, and by kissing. Finding the most important risk factors that contribute to the spread of typhoid fever is the primary goal of this investigation. The technique known as Topsis Multi Criteria Decision Making (MCDM) will be employed to ascertain the extent of typhoid fever outbreak. These days, MCDM technologies are used to environmental, social, and economic issues, among others (5-7). Certain MCDM strategies assign a rank of indications, while others assign a priority value. Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS), put forth by Hwang and Yoon in 1981 (8), is one of the ranking-based MCDM tools.

**Model of Topsis**

The Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS), which analyzes decisions for alternatives, is one of the many criteria approaches. The TOPSIS notion is logical and comprehensible, and the computation required is simple. It's also important to acknowledge the inherent difficulties in determining trustworthy subjective preferences for the criterion (9). The TOPSIS technique flow chart is shown in Figure 1.

Decision-making in TOPSIS is essentially based on six ineffective steps:

(i) Determine the decision matrix's normalized values.

Think about the fact that there are m alternatives. D = [rij]m×m be the normalized matrix. The definition of the normalized value rij is

$$rij = \frac{xij}{\sqrt{\sum_{i=j}^{m} xij2}} \forall\, i\,, j$$

(ii) Determine the decision matrix that is weighted and normalized. It is calculated to get the weighted normalized value vij as

$$\sum_{i=1}^{n} W_j = 1$$

$vij = wjrij, \forall i,j$

where wj is the jth criterion's weight, and

(iii) Find the optimal and non-optimal solution

$A^{+} = \{v1+, v2+, v3+, \ldots, vm+\} = \{(\max ivij \mid j \in Cb), (\min ivij \mid j \in Cc)\}$

$A^{-} = \{v1-, v2-, v3-, \ldots, vm-\} = \{(\min ivij \mid j \in Cb), (\max ivij \mid j \in Cc)\}$

Where Cc is linked to the cost criteria and Cb to the benefit criteria.

(iv) Use the m dimensional supremum distance to compute the separation metrics. We use the Euclidean distance to compute separation metrics in TOPSIS. The following represents how each option and its negative relation to the ideal answer are separated:

$S_i^{+} = \max\{|vij - vj+| : j = 1, 2, \ldots, m\}, \forall i$

$$S_i^{-} = \sqrt{\sum_{j=1}^{m} (\mathrm{vij} - \mathrm{vj-})^2}, \forall\ \mathrm{i}$$

(v) Determine how near the optimal answer you are:
The definition of the alternative Ai's relative proximity to $A-$ is

$$RC_i^* = \frac{S_i^-}{S_i^- + S_i^+}, \forall \text{ i}$$

(vi) Determine the order of preference. The range of $RCi*$ index values is 0 to 1. The closer an alternative is to the optimal answer, the higher the index value.

## METHODOLOGY

The primary goal of this study is to determine the most important risk factor for the spread of typhoid fever.
Let E = f(D,W) denote the risk of spread of Typhoid fever.
Where,
D stands for the collection of all of E's elements.
D = {M : M = all selected factors}
W = {W : W = Weights all selected}
Where M and W stand for the parameter that has been chosen and its PV, respectively.
The current study's methodology is broken down into three sections: using the Topsis technique, choosing alternatives, and selecting criteria.

**Selection of Criteria**
The chosen literature review, expert survey, and media survey are the study's current criteria. In order to identify every risk factor for the spread of typhoid fever, we examined close to thirty publications. Therefore, the literature review is a key selection criterion for all of the choices in this study. Since the study's focus is on medical issues, experts play a significant role. To determine the risk factors for the spread of typhoid fever, we gathered the opinions of about ten specialists. Another crucial criterion for factor selection is the media survey, since they are constantly seeking to gather data regarding typhoid fever. In this study, the risk factor is chosen based on reports from three credible media outlets.

**Selection of Alternatives**
Expert survey and relative peasant opinions are taken into consideration as criteria in this study's literature evaluation, and they are represented by the letters C1, C2, and C3, respectively. Typhoid fever risk factors are shown in Table 1. The decision hierarchy is displayed in Figure 2. Our decision problem's structure. A five-point rating system Table 3 convert as a score and score table represented by Table 4. The formula represents each of Table 4's column vectors. Following computation, the normalized decision matrix shown in Table 5 is the result. Table 5 shows the PV for each criterion as determined by the MCDM approach in column two.

$$rij = \frac{xij}{\sqrt{\sum_{i=1}^{4} x_{1j}^2}}, \forall\, i = 1,2,3, j = 1,2,3$$

Compute the weighted normalized decision matrix from Table 5 using the formula. The weighted normalized decision matrix is shown in Table 6. Utilize the formulas in (2) and (3) to find the ideal A+ and negative-ideal A-solution. Table 6's final two columns show the A+ and A-values.

$vij = wjrij, \forall i = 1,2,3, j = 1,2,3.$ (1)

$A+ = \{v1+, v2+, v3+, \ldots, vm+\} = \{(maxivij \mid j \in Cb)\}$ (2)

$A- = \{v1-, v2-, v3-, \ldots, vm-\} = \{(minivij \mid j \in Cb)\}$ (3)

**$RC_i$* value for Topsis**

| | $S_i^+$ | $S_i^-$ | $S_i^+ + S_i^-$ | RCi* | Rank |
|---|---|---|---|---|---|
| $A_1$ | 0 | 0.24165459 | 0.24165459 | 1 | 1 |
| $A_2$ | 0.12081266 | 0.12082905 | 0.24164171 | 0.5 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| $A_3$ | 0.24165817 | 0 | 0.24165817 | 0 | 3 |

## CONCLUSION

With the aid of the Topsis MCDM approach, the current study has sought to create a novel model for evaluation of the risk assessment of typhoid fever. This new model will have the advantage of being able to study the spread of typhoid fever via doctors' opinions, literature reviews, and media surveys in an objective and cognitive manner. We have identified three potential risk factors for typhoid fever: bacterial contamination, contact with an infected individual, and kiss-related contamination. Following the gathering of all the necessary data, we used Fuzzy Topsis MCDM to determine the PV of the criteria. Utilizing the PV of the criterion, use Topsis to identify the optimal options. Finally, through media surveys, I concluded using the Topsis MCDM method that Typhoid fever is more spread due to sewage contamination.

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**Table 1: Explanation of Chosen Elements**

| Name of Element | Description | Reference | |
|---|---|---|---|
| Contamination due to bacteria (A1) | The bacterial spread of sewage contamination of food or water. | (2,24) | |
| Contact with an infected individual resulting in contamination (A2) | There is a significant likelihood of the virus spreading through contact with an infected individual. People are asked to maintain safe distances because of this. | (2,24) | |
| Contamination due to kiss (A3) | If kiss to loved one in your families and relatives there is a chance to spread of this virus. | (2,25,26) | |

**Table 2: 5-Point Scale**

| Name | Score |
|---|---|
| Average | 1 |
| Good | 2 |
| Very Good | 3 |
| Excellent | 4 |
| Outstanding | 5 |

**Table 3:** Table of scores for the alternatives based on the criteria

| Alternatives | Criteria | | |
|---|---|---|---|
| | C1 | C2 | C3 |
| A1 | V.G | E | O |
| A2 | G | V.G | E |
| A3 | A | G | V.G |

**Table 4:** Using a 5-point rating system, create an alternate score table.

| Alternatives | Criteria | | |
|---|---|---|---|
| | C1 | C2 | C3 |
| A1 | $X_{11} = 3$ | $X_{12} = 4$ | $X_{13} = 5$ |
| A2 | $X_{21} = 2$ | $X_{22} = 3$ | $X_{23} = 4$ |
| A3 | $X_{31} = 1$ | $X_{32} = 2$ | $X_{33} = 3$ |
| $\sqrt{\sum_{i=1}^{3} x_{ij}^2}$ | 3.74165739 | 4.35889894 | 7.07106781 |

**Table 5. Decision Matrix Normalized**

| | Priority ranking for every criterion | | |
|---|---|---|---|
| | $w_1 = 0.2592593$ | $W_2 = 0.3333333$ | $W_3 = 0.4444444$ |
| Alternatives | C1 | C2 | C3 |
| A1 | $r_{11} = 0.80178373$ | $r_{12} = 0.91766294$ | $r_{13} = 0.91766294$ |
| A2 | $r_{21} = 0.53452248$ | $r_{22} = 0.68824720$ | $r_{23} = 0.68824720$ |
| A3 | $r_{31} = 0.26726124$ | $r_{32} = 0.45883147$ | $r_{33} = 0.45883147$ |

**Table 6. Normalized Weighted Decision Matrix**

| Alternatives | C1 | C2 | C3 |
|---|---|---|---|
| A1 | $v_{11} = 0.20786989$ | $v_{12} = 0.30588762$ | $r_{13} = 0.31426965$ |
| A2 | $v_{21} = 0.13857992$ | $v_{22} = 0.22941511$ | $r_{23} = 0.25141572$ |

| | | | |
|---|---|---|---|
| A3 | $v_{31}$ = 0.06928996 | $v_{32}$ = 0.15294381 | $r_{33}$ = 0.18856179 |
| $A^+$ | $v^+_1$ = 0.20786989 | $v^+_2$ = 0.30588762 | $v^+_3$ = 0.31426965 |
| $A^-$ | $v^-_1$ = 0.06928996 | $v^-_2$ = 0.15294381 | $v^-_3$ = 0.18856179 |

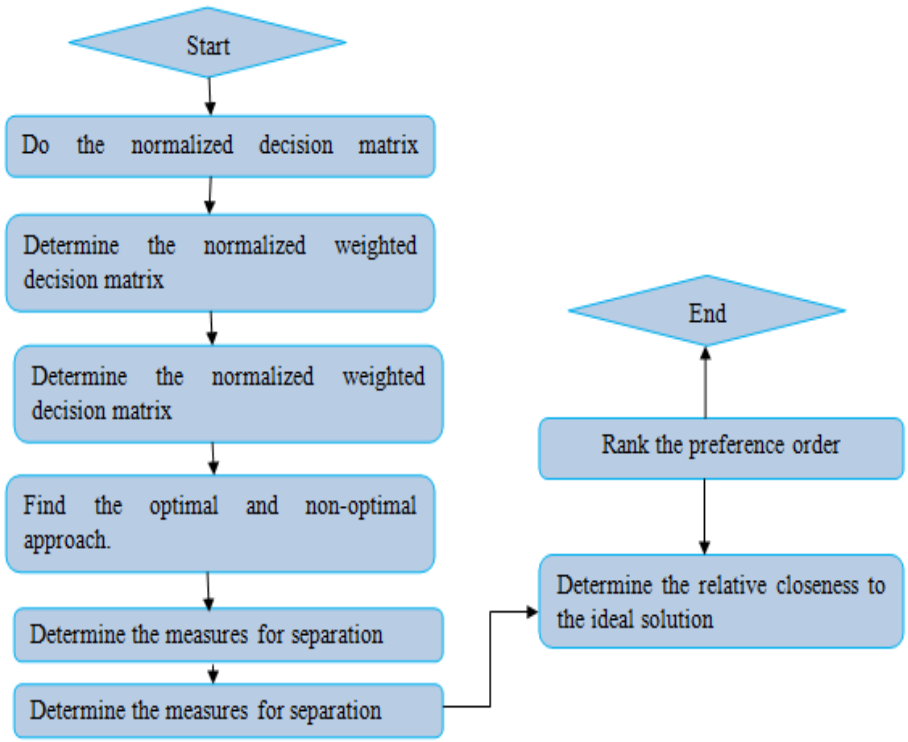


**Figure 1. The TOPSIS technique flow chart**

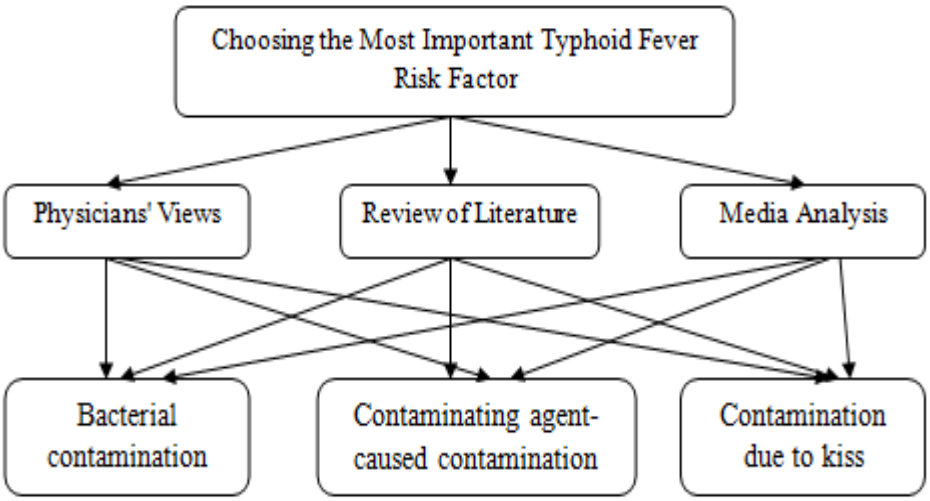


**Figure 2. Decision hierarchy Structure**

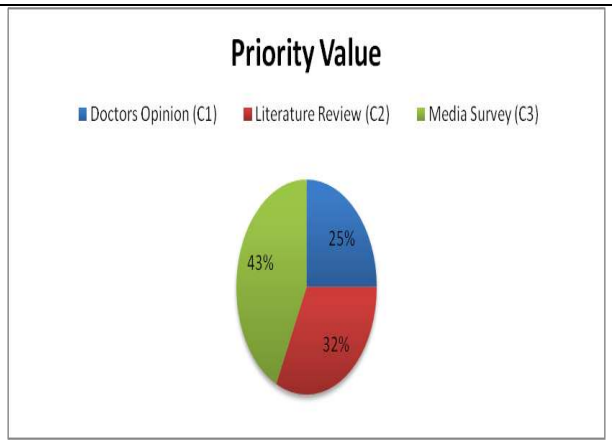


**Figure 3. Outcome based on Fuzzy MCDM Criteria**

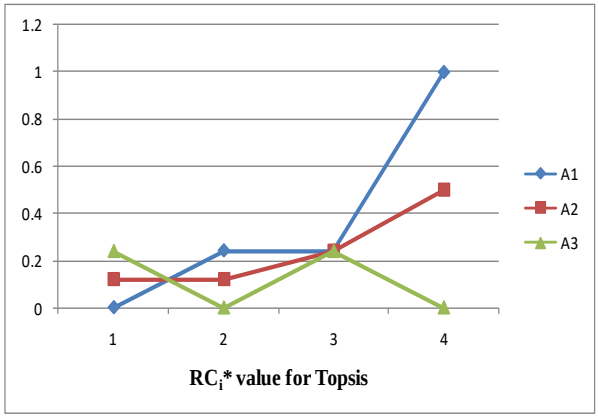


**Figure 4. RCi* value for Topsis**

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**RESEARCH ARTICLE**

# Unveiling the Cloud Kitchen Phenomenon: Analyzing Customer Awareness and Shifting Preferences

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**ABSTRACT**

The research explores the transformative impact of cloud kitchens in the food service industry. It aims to understand the necessity of cloud kitchens and discern how they differ from traditional dine-in establishments. The study also investigates key factors influencing consumer decisions, such as purchasing behavior, perceptions, and challenges faced when ordering from cloud kitchens. The research employs a descriptive research design, utilizing both primary and secondary data sources. Primary data is collected through a questionnaire method, focusing on Ahmedabad customers. Secondary data is sourced from internet-available reports and journals. The major purpose of the study is to ensure a holistic understanding of the evolving landscape of cloud kitchens. The study reveals a growing awareness of cloud kitchens, primarily driven by social media platforms. Factors influencing consumer decisions include online presence, food quality, and affordability. Cloud kitchens, operating as production units with streamlined costs, offer benefits like faster delivery, sustainability, and an alternative to traditional dining. The findings suggest practical implications for stakeholders in the food service industry. Cloud kitchens can enhance their visibility on social media platforms to capitalize on the growing awareness. The identified benefits, including faster delivery and sustainability, provide practical insights for cloud kitchen operators aiming to meet consumer expectations. This research contributes to the understanding of the dynamic landscape shaped by cloud kitchens. The study's contribution lies in its comprehensive analysis, shedding light on the transformative role of cloud kitchens in the evolving food service industry.

**Keywords:** Cloud Kitchen, Customer Preference, Consumer Behavior, Food Service Industry

## INTRODUCTION

In the ever-evolving landscape of the food service industry, a revolutionary concept has emerged, challenging traditional norms and redefining how individuals experience and access food – the cloud kitchen. This innovative model operates as a production unit focused solely on preparing and delivering food, eliminating the need for expensive front-of-house operations. Its key features are as follows:

**Breaking Free from the Bricks and Mortar**

Unlike conventional restaurants with dine-in facilities, cloud kitchens operate with a leaner business model, offering several advantages. Firstly, they significantly reduce overhead costs by eliminating expenses related to rent, furniture, décor, and tableware. This reduction in fixed costs translates to improved profit margins for cloud kitchens. Additionally, cloud kitchens boast increased flexibility, allowing them to scale up or down depending on demand, adapt to market conditions, and test new concepts. The absence of dine-in operations also enhances their focus on quality and efficiency in food preparation.

**Catering to the Digital Appetite**

The rise of cloud kitchens is closely tied to the growing popularity of online food delivery platforms. By partnering with these platforms, cloud kitchens gain access to a vast network of potential customers, tapping into the ever-increasing demand for convenient and affordable home delivery. This partnership not only facilitates broader market reach but also provides valuable data and insights into consumer preferences. With this information, cloud kitchens can tailor their menus and promotions to specific customer segments, enhancing their appeal in a competitive market.

**Diverse Culinary Landscape**

Initially associated with fast food and quick-service meals, cloud kitchens have evolved to encompass a diverse range of culinary experiences. Modern cloud kitchens now offer everything from gourmet burgers and handcrafted pizzas to vegan dishes, ethnic cuisines, and healthy meal plans. This diversity caters to the increasingly sophisticated palates of modern consumers, allowing cloud kitchens to tap into niche markets and cater to various dietary preferences. This adaptability positions cloud kitchens as dynamic players in the culinary scene.

**Innovations and Future Potential**

Cloud kitchens are not mere trends; they represent a paradigm shift in the food service industry. As technology continues to advance, further innovations in this space are expected. Automation, including robotic kitchens and automated order fulfilment systems, holds the promise of improving efficiency and reducing labor costs. Artificial intelligence (AI) is poised to play a significant role in personalizing menus, recommending dishes, and optimizing delivery routes, enhancing the overall customer experience. Data-driven decision-making, informed by customer data and trends, empowers cloud kitchens to make strategic choices regarding menu development, pricing strategies, and marketing campaigns.

**The Impact on the Food Service Landscape**:The rise of cloud kitchens is reshaping the traditional food service industry. While some traditional restaurants may face challenges adapting to this new landscape, others are embracing the concept by launching their virtual kitchens to expand their reach and cater to the growing demand for delivery. This coexistence of traditional and virtual models is fostering a more dynamic and competitive food service landscape, ultimately benefiting consumers with a wider variety of choices and convenient access to delicious meals.

## LITERATURE REVIEW

The studies conducted by Mathur (2023) and Deepak (2022) shed light on the evolving landscape of food consumption, particularly the rise of cloud kitchens. The study (Mathur, 2023)emphasized the strong influence of perception on consumer purchase intentions, providing valuable insights for cloud kitchen businesses. Deepak(2022) delved into the operational dynamics of cloud kitchens, emphasizing their exclusive focus on food delivery and the cost advantages compared to traditional setups. Through regression analysis and customer surveys, the study (Deepak, 2022)explored factors influencing cloud kitchen businesses. During the COVID-19 pandemic, cloud kitchens played a pivotal role, as outlined in the study (Rudrani Chatterjee, 2022). This research explored distinctive aspects, consumer behavior, and the pandemic's impact on food delivery interactions. It sought to identify challenges and consumer preferences, contributing valuable insights for the industry's future growth. Shaik Mehnaz et al. (2021)

provided an analysis of cloud kitchens in India, offering insights into industry trends and impacts, particularly during the pandemic. The paper emphasized the disruptive nature of the industry and its evolving landscape(Shaik Mehnaz, 2021).The study by Sanjukta surge in online food delivery's popularity in India, driven by lifestyle changes and mobile technology. The study identified factors affecting consumer adoption of cloud kitchens, emphasizing the significance of facilitating conditions, social influence, online coupons, and food variety(Sanjukta Pookulangara, 2023).

The study (Han-Shen Chen, 2020) and (Nikhil Chhabra, 2021)analyzed consumer perceptions of online food ordering services in India, aiming to understand the factors attracting consumers to these services.Also cloud kitchen-based studies are conducted in other countries like Hakim explored consumerawareness of cloudkitchen in Brazil (Hakim, 2022). Dsouza (2022) identified factors influencing customers' intentions to use mobile apps for ordering food, revealing the positive effect of six major factors(Dsouza Prima Frederick, 2022). Sharma (2023) examined the structural relationship between various factors influencing behavioral intention toward online food delivery services (Sharma, 2023). Nurul (2023) investigated the influence of consumer behavioral intention toward online food delivery services, providing insights into key factors affecting consumers' intentions(Nurul Syahirah Idris, 2023).

## RESEARCH METHODOLOGY

This study aims to investigate the necessity of cloud kitchens and explore customer preferences between traditional dine-in establishments and cloud kitchens. The objectives include assessing the purchasing behavior from cloud kitchens, understanding customer preferences, identifying factors influencing buying decisions, and examining challenges faced by customers when ordering from cloud kitchens. The scope of the study focuses on examining the awareness level of cloud kitchens among customersand exploring their preference for cloud kitchens. The research design is descriptive, employing both primary and secondary data sources. Primary data is collected through a questionnaire method from customers in Ahmedabad, while secondary data from reports and journals available on the internet supplements the study. The sampling plan targets all customers in Ahmedabad, with the sampling units being customers aware of cloud kitchens. The study includes a sample size of 152 customers, selected through non-probability convenience sampling. The research instrument is a questionnaire, and data analysis employs SPSS software.

**Analysis & Interpretation**

The chi-square test was conducted to analyze the association between age groups and the purchase from cloud kitchens. The crosstabulation (Table 1) presents a breakdown of respondents based on their ageand whether they have purchased from cloud kitchens. The data reveals that the majority of respondents in the 26-35 age group have purchased from cloud kitchens, with 48 respondents affirming their purchases compared to 9 who have not. This is followed by the 18-25 age group, with 39 respondents indicating they have purchased from cloud kitchens.

The chi-square test statistic is calculated as 8.634 with 3 degrees of freedom, resulting in an asymptotic significance value of 0.035 (Table 2). The null hypothesis (H0) posits that there is no association between age and purchasing habits from cloud kitchens, while the alternative hypothesis (H1) suggests a significant association. The critical p-value for rejecting the null hypothesis is conventionally set at 0.05.

Given that the calculated p-value (0.035) is less than 0.05, the null hypothesis is rejected. This indicates a statistically significant association between age groups and the likelihood of purchasing from cloud kitchens. Therefore, it can be concluded that age influences purchase intentions from cloud kitchens. The 26-35 age group showed the highest propensity to buy from cloud kitchens, primarily comprising of Millennials who are likely to be the part of working population. The rejection of the null hypothesis suggests that there is a notable association between age and the decision to purchase from cloud kitchens, emphasizing the importance of considering age demographics whileanalyzing consumer behaviortowards cloud kitchens.Also, the chi-square test was conducted to assess the

relationship between marital status and the frequency of ordering from cloud kitchens within a month. The crosstabulation (Table 3) presents a breakdown of respondents based on their marital status (Single or Married) and ordering frequency.

The data shows that among single individuals, 34 respondents rarely ordered from cloud kitchens, 24 occasionally ordered, and 6 frequently ordered. For married individuals, 20 respondents rarely ordered, 26 occasionally ordered, and 3 frequently ordered. The chi-square test statistic is calculated as 3.403 with 3 degrees of freedom, resulting in an asymptotic significance value of 0.334 (Table 4).The null hypothesis (H0) posits that there is no relationship between marital status and the frequency of ordering from cloud kitchens, while the alternative hypothesis (H1) suggests a significant relationship. The critical p-value for rejecting the null hypothesis is conventionally set at 0.05. However, in this case, the calculated p-value (0.334) is greater than 0.05, leading to the acceptance of the null hypothesis.
Therefore, the results indicate that there is no statistically significant association between marital status and the frequency of ordering from cloud kitchens within a month. In other words, marital status does not appear to influence how frequently individuals order from cloud kitchens. This suggests that factors other than marital status may play a more prominent role in determining the ordering habits of individuals from cloud kitchens

The factor analysis conducted on the dataset aimed to explore underlying patterns and relationships within the variables related to consumer preferences and buying behavior towards cloud kitchens. The reliability test indicated that the overall data is highly reliable, with a Cronbach's Alpha value of 0.889, surpassing the recommended threshold of 0.7.The KMO and Bartlett's Test provided further insights into the suitability of the data for factor analysis. With a KMO value of 0.878, which is considered excellent (above 0.8), and a significant Bartlett's Test ($p < 0.05$), it was determined that the dataset is well-suited for factor analysis.The Total Variance Explained (Table 5) displayed those four components had eigenvalues greater than 1, covering a cumulative variance of 67%. This surpasses the standard recommendation of 60%, indicating that the extracted factors explain a substantial portion of the total data variance.

The Rotated Component Matrix (Table 6) revealed the statements associated with each of the four extracted factors. The factors were labelled as follows: 1) Digital Culinary Experience 2) Influential Cloud Kitchen Acumen 3) Cloud Kitchens Ordering Ease and 4) Socialized Culinary Advocacy. The Nomenclature Table (Table 7) provided a clear overview of how statements were grouped under each factor. Furthermore, the Reliability Test of All the Factors (Table 8) assessed the internal consistency of each factor. All four factors demonstrated high reliability, with Cronbach's Alpha values exceeding 0.7, reinforcing the robustness of the extracted factors. In summary, the factor analysis successfully identified and labelled four key factors that collectively explain a significant proportion of the variance in the data. These factors represent distinct dimensions of customer preference and buying behavior related to cloud kitchens, providing valuable insights for understanding the multifaceted nature of customer preference towards this emerging culinary concept of cloud kitchen.

## FINDINGS

The study aimed to achieve two primary objectives: understanding the customers buying behavior from cloud kitchens and gauging their preferences toward this emerging food service model. Regarding the first objective, it was found that 76% of the respondents had purchased from cloud kitchens, with the remaining 24% citing reliability issues, often related to quality or trust concerns, as the primary reason for not doing so. This finding reflects higher level of awareness about cloud kitchens. Moreover, a significant portion, 54%, ordered from cloud kitchens 1-2 times a month indicating higher preference of ordering from cloud kitchen. Price and affordability emerged as the most influential factor during the purchasing decision, followed by the quality of food and online presence. Respondents were least influenced by the speed of delivery. Social media platforms and word of mouth were the predominant channels through which respondents became aware of cloud kitchens. The most significant challenges reported included the limited availability of cloud kitchens in the market and insufficient customer assistance.

In pursuit of the second objective, the study delved into customer preference towards cloud kitchens. Factors such as digital culinary experience, socialized culinary advocacy, ordering ease offered by cloud kitchens played a crucial role in justifying the shifting preference towards cloud kitchens. Enhanced awareness and improved customer service were identified as critical areas requiring attention. Notably, 14% of respondents did not view cloud kitchens as the future, while 58% expressed a willingness to continue using them. A significant portion, 50%, would recommend cloud kitchens to others. Gender-wise, more males believed in the future of cloud kitchens than females. The age group of 26–35 years emerged as the one making frequent purchases among all age groups, highlighting potential target demographics forformulating cloud kitchen related marketing strategies. Overall, the study provides valuable insights into both the consumer behavior and preference surrounding cloud kitchens.

## CONCLUSION

The Indian Cloud Kitchen Market has experienced significant expansion and is projected to grow at a rate of 24% by 2026 (ETRetail, 2023).The study reveals a growing awareness of cloud kitchens, indicating the need for increased visibility through social media platforms. Benefits associated with cloud kitchens such as faster delivery, sustainability, and a viable alternative to traditional dining are the major reasons contributing towards its wider acceptability. The food market is poised for growth in the coming years due to rising disposable income, changing lifestyles, and a busy work culture. In this scenario, it is worth noting that despite being a relatively new concept, customers willingness to continue using cloud kitchens in their daily lives, highlights a positive and progressive outlook for the industry in the coming years.

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**Table 1 Crosstab of Age & Purchase from Cloud Kitchens**

| | | Have you purchased from Cloud Kitchen | | Total |
|---|---|---|---|---|
| | | Yes | No | |
| Age | 18-25 | 39 | 10 | 49 |
| | 26-35 | 48 | 9 | 57 |
| | 36-45 | 22 | 10 | 32 |
| | 46 & above | 7 | 7 | 14 |
| **Total** | | **116** | **36** | **152** |

**Table 2 Chi-Square Tests**

| | Value | Df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 8.634[a] | 3 | .035 |

**Table 3 Crosstab of Marital Status & Ordering Frequency from Cloud Kitchens**

| | | How Frequently do you Order from Cloud Kitchen within a Month? | | | | Total |
|---|---|---|---|---|---|---|
| | | Only Heard of Cloud Kitchens but never Ordered | Rarely (1-2 times) | Occasionally (3-5 times) | Frequently (6 or more times) | |
| Marital Status | Single | 1 | 34 | 24 | 6 | 65 |
| | Married | 2 | 20 | 26 | 3 | 51 |
| **Total** | | **3** | **54** | **50** | **9** | **116** |

**Table 4Chi-Square Test**

| | Value | Df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 3.403a | 3 | .334 |

**Table 5Total Variance Explained**

| Component | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | | Rotation Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 6.783 | 42.396 | 42.396 | 6.783 | 42.396 | 42.396 | 3.588 | 22.424 | 22.424 |
| 2 | 1.719 | 10.745 | 53.141 | 1.719 | 10.745 | 53.141 | 2.656 | 16.600 | 39.024 |
| 3 | 1.125 | 7.032 | 60.173 | 1.125 | 7.032 | 60.173 | 2.348 | 14.677 | 53.701 |
| 4 | 1.014 | 6.338 | 66.511 | 1.014 | 6.338 | 66.511 | 2.050 | 12.810 | 66.511 |
| 5 | .811 | 5.066 | 71.577 | | | | | | |
| 6 | .719 | 4.496 | 76.073 | | | | | | |
| 7 | .621 | 3.881 | 79.953 | | | | | | |
| 8 | .559 | 3.491 | 83.444 | | | | | | |
| 9 | .506 | 3.162 | 86.606 | | | | | | |
| 10 | .395 | 2.468 | 89.074 | | | | | | |
| 11 | .389 | 2.429 | 91.503 | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 12 | .381 | 2.379 | 93.881 | | | | | | |
| 13 | .293 | 1.830 | 95.711 | | | | | | |
| 14 | .273 | 1.705 | 97.416 | | | | | | |
| 15 | .227 | 1.416 | 98.832 | | | | | | |
| 16 | .187 | 1.168 | 100.000 | | | | | | |

**Table 6 Rotated Component Matrix**

| Statements | Component | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Cloud Kitchen perform better than Traditional Restaurants | 0.788 | | | |
| The shift to cloud kitchens is beneficial for the society | 0.74 | | | |
| Cloud Kitchen Concept attracts me | 0.697 | | | |
| Cloud Kitchens offer better value for money compared to traditional restaurants. | 0.678 | | | |
| I trust the quality of food from Cloud Kitchens. | 0.673 | | | |
| Cloud Kitchens offer a convenient way to order food. | 0.607 | | | |
| I have positive feeling when ordering and buying from cloud kitchens | 0.529 | | | |
| I am interested in knowing more about Cloud Kitchens | | 0.749 | | |
| Social media platforms and online advertisements have influenced my awareness and perception of Cloud Kitchens. | | 0.697 | | |
| Online reviews and personal recommendations are significant factors in my perception of Cloud Kitchens. | | 0.671 | | |
| I am more likely to order from Cloud Kitchens when they provide attractive deals and discounts. | | 0.648 | | |
| I frequently use food delivery apps to order from Cloud Kitchens. | | | 0.803 | |
| I frequently order from Cloud Kitchens. | | | 0.71 | |
| Price is a significant factor in my decision to order from Cloud Kitchens. | | | 0.696 | |
| My friends often share their Ordering experience from Cloud Kitchens | | | | 0.85 |
| My friends often recommend to order from Cloud Kitchens | | | | 0.819 |
| Extraction Method: Principal Component Analysis. | | | | |
| Rotation Method: Varimax with Kaiser Normalization. | | | | |

**Table 7 New Nomenclature**

| No. | Factorial Group | Statements |
|---|---|---|
| 1 | Digital Culinary Experience | Cloud Kitchen perform better than Traditional Restaurants |
| | | The shift to cloud kitchens is beneficial for the society |
| | | Cloud Kitchen Concept attracts me |
| | | Cloud Kitchens offer better value for money compared to traditional restaurants. |

| | | |
|---|---|---|
| | | I trust the quality of food from Cloud Kitchens. |
| | | Cloud Kitchens offer a convenient way to order food. |
| | | I have positive feeling when ordering and buying from cloud kitchens |
| 2 | Influential Cloud Kitchen Acumen | I am interested in knowing more about Cloud Kitchens |
| | | Social media platforms and online advertisements have influenced my awareness and perception of Cloud Kitchens. |
| | | Online reviews and personal recommendations are significant factors in my perception of Cloud Kitchens. |
| | | I am more likely to order from Cloud Kitchens when they provide attractive deals and discounts. |
| 3 | Cloud Kitchens Ordering Ease | I frequently use food delivery apps to order from Cloud Kitchens. |
| | | I frequently order from Cloud Kitchens. |
| | | Price is a significant factor in my decision to order from Cloud Kitchens. |
| 4 | Socialized Culinary Advocacy | My friends often share their Ordering experience from Cloud Kitchens |
| | | My friends often recommend to order from Cloud Kitchens |

**Table 8 Reliability Test of Extracted Factors**

| No. | Factorial Group | Cronbach's Alpha |
|---|---|---|
| 1 | Digital Culinary Experience | 0.867 |
| 2 | Influential Cloud Kitchen Acumen | 0.805 |
| 3 | Cloud Kitchens Ordering Ease | 0.765 |
| 4 | Socialized Culinary Advocacy | 0.859 |

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**REVIEW ARTICLE**

# A Review on Drug-Drug Interaction and Pharmacokinetic Studies of Metformin and Furosemide with Other Drugs.

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**ABSTRACT**

Drug-drug Interactions is a significant therapeutic concern, especially when administering many medications to patients with conditions like diabetes, hypertension and other cardiovascular diseases. In case of diabetes, metformin is more commonly prescribed drug, it is a derivative of biguanide used to treat type-2 diabetes. Similarly, in case of hypertension, furosemide is more commonly prescribed drug, it is a loop diuretic usedin the treatment of edema state associated with chronic failure, hypertension, heart failure and liver cirrhosis. Hence, our aim is to know the DDI of metformin alone or with other drugs like, Chiglitazar, Pioglitazone, Ranolazine and telmisartan etc and DDI of furosemide alone or with other drugs like, Tolvaptan, spironolactone etc. In this review article, we were discussed the DDI that was performed by using a high-pressure liquid chromatography with different detectors and different species were used to study the drug-drug interaction of metformin with other drugs and furosemide with other drugs and different study design were applied to know the pharmacokinetic of the drugs

**Keywords:** Metformin, Furosemide, different chromatographic condition and species, drug-drug interaction and Pharmacokinetic studies.

## INTRODUCTION

The evaluation of possible drug-drug interactions (DDIs) has grown in significance due to people's frequent concurrent usage of many medicines. Many people have employed physiologically based pharmacokinetic modelling techniques to quantitatively estimate drug-drug interactions (DDIs) between therapeutic medicines that are delivered

together[1].Pharmaceutical, toxicological, doping and clinical chemistry research all depend on the identification and measurement of substances in biological fluids. The concentration of medications in biological fluids and tissues is correlated with therapeutic efficacy[2]. In this article we are going to study about drug-drug interaction and pharmacokinetic studies on Metformin and Furosemide with other drugs. Type-2 diabetes mellitus is a chronic disease characterized by decrease in the pancreas' ability to release insulin, a resistance to insulin in peripheral tissues, or both[19]. A diabetic patient can maintain stable blood glucose level with the aid of antidiabetic medications, a nutritious diet, and regular exercise[17,21]. The first oral hypoglycaemic medication, metformin (MET) is a biguanide derivative, lowers plasma glucose levels by improving insulin sensitivity and reducing hepatic glucose release[3,26]. Metformin reduces insulin resistance and hepatic glucose production while increasing peripheral glucose absorbance and insulin sensitivity[4]. Additionally, it had a therapeutic impact on obesity metabolic disease by lowering free fatty acids (FFA). Sometimes, women with PCOS (polycystic ovary syndrome) depend on this medication for regular menstrual cycles and ovulation[5]. Metformin is also used to manage the cardiac hypertrophy, cardiac fibrosis, regulation of lipid[18], cancer and aging[24].

Diuresis-promoting medications are frequently used to treat edematous disorders, where an increase in urine flow reduces symptoms[6,23]. Several medical conditions are also treated by using diuretics which includes hypertension, liver cirrhosis, cardiac and renal failure[15,22,25]. Diuretics are drugs that cause the body's sodium that is bonded to anions and water to be excreted more in urine, which lowers the concentration of sodium in extracellular liquids. Effective diuretic like, Furosemide (FU) is commonly used to treat edema, fluid accumulation in body cavities[16], liver cirrhosis[29], hypertension, chronic renal failure, and congestive heart failure[20,23]. Due to FUR's primary action in the nephron, where it prevents sodium from being tubularly reabsorbed on Henle's loop, the medication is frequently categorized as a loop diuretic. FUfunctions by preventing the co-transport of potassium, sodium, and chloride and also excretes bicarbonate, calcium, and magnesium ions[31]. By lowering the levels of other doping drugs in urine, intense and quick dieresis may also cover up the use of other substances[6].

**Mechanism of action of Metformin**

Metformin improves insulin sensitivity by minimizing peripheral glucose uptake and utilization, gluconeogenesis and intestinal glucose absorption[27,32]. Orally administered Metformin enter into the hepatocytes via the portal vein, where it is taken up by OCT1 plasma membrane transporters. Metformin inhibits the action of mitochondrial respiratory chain complex-1 in the cell, which raises AMP and decreases ATP levels[33]. Raised AMP levels trigger the activity of Adenosine Monophosphate-Activated Protein Kinase (AMPK), which inhibits the production of glucose through at least two mechanisms: The first is that elevated AMPK phosphorylates the transcription factors CBP and CRTC2, which inhibits the genes that produce glucose (also known as "gluconeogenic genes"); Secondly, elevated AMPK also inhibit the function of mGPD by increasing cytosolic NADH and promoting the conversion of pyruvate to lactate, while simultaneously reducing gluconeogenesis who also have other diseases that result in metabolic acidosis may accumulate lactate to harmful levels (lactic acidosis) or Patients who use metformin and have other health conditions that cause metabolic acidosis may accumulate lactate to fatal amounts[7].

**Mechanism of action of Furosemide:**

Furosemide is a loop diuretic having the ability to block the Na+, K+ and 2Cl- symporters in the loop of Henle's thick ascending limb[28,30,34]. Consequently, raises the excretion of Ca2+, Mg2+, Na+, K+ and Cl- in the urine[8].Like other loop diuretics, Furosemide lowers the excretion of uric acid. It immediately dilates the blood arteries that helpful for treating acute pulmonary edema[35]. Vasodilation reduces the creation of endogenous natriuretic hormonesthat have the ability to constrict blood vessels and reduces the body's susceptibility to vasoconstrictors like noradrenalin and angiotensin II[7]. Few analytical techniques have been documented to date for the determination of metformin in biological fluids, either by itself or in combination with other antidiabetic medications, according to a review of the literature[3] Nowadays people with diabetes also suffer from additional chronic conditions like nephropathy and hypertension and other heart related disease[40,41]. When metformin with other drugs is used together, there may be drug-drug interaction takes place. An improper dose modification might result in lactic acidosis, which can be fatal[9,36]. Normally, this has little or no effect on the buildup of lactate at therapeutic doses of metformin. Lactate

absorption by the liver is decreased and lactic acidosis results if metformin accumulates as a result of either poor elimination or excessive ingestion[38]. Metformin-associated lactic acidosis (MALA) is caused by an imbalance between the amount of lactate produced and the decreased amount of lactate eliminated[37,39]. Metformin is removed by renal tubular secretion, several drugs that are cationic agents may compete with it for elimination. If taken concurrently, several of them—such as digoxin, quinidine, ranitidine, furosemide or nifedipine-may raise the level of metformin. Therefore, it's critical to keep on the lookout for any potential toxicity in individuals taking metformin in combination with these drugs[10].

## METHODOLOGY

**Drug-drug interaction of Metformin with other drugs:**
Show the Table 1: Pharmacokinetic analysis of metformin alone and in combination with other compounds using high-performance liquid chromatography (HPLC) with different species.
**Drug-drug interaction of furosemide with other drugs:**
Show the Table 2: Pharmacokinetic analysis of furosemide alone and in combination with other compounds using HPLC in various species to determine drug-drug interactions.
**Outcomes of the Studies**
**Pharmacokinetic parameters of Metformin-Chiglitazar**
Show the Table 3: Pharmacokinetic parameters of Metformin, Chiglitazar alone and in combination[11].

A study on the drug-drug interaction between Chiglitazar and Metformin was carried out by Ling Yi and Hua Zhang. They found that pharmacokinetic characteristics like $t_{max}$ and $t_{1/2}$ do not vary appreciably when the two medications are taken concurrently. Metformin raises the CL/F of Chiglitazar while lowering the $C_{max}$, $AUC_{0-48h}$, and $AUC_{0-\infty}$. Chiglitazar increases the $AUC_{0-48h}$, $AUC_{0-\infty}$, and $C_{max}$ of metformin while decreasing the $t_{1/2}$ and CL/F[11].

**Pharmacokinetic parameters of Metformin-Pioglitazone:**
Show the Table 4: Pharmacokinetic parameters of Metformin, Pioglitazone alone and in combination[3].
In the study of drug-drug interaction between pioglitazone and metformin, Mohamed Saleh Elgawish and Sally Nasser observed that when metformin and pioglitazone were administered together, PGT (50 mg/kg) reduced the pharmacokinetic parameter such as $C_{max}$, $t_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$ and Kel of metformin but increased Vd/F and CL/F of metformin in contrast to MET alone. Metformin (50 mg/kg) also reduces $AUC_{0-t}$, $AUC_{0-\infty}$, Kel and $C_{max}$ , but it raises PGT's $t_{max}$, $t_{1/2}$, Vd/F and Cl/F when given with PGT[3].
**Pharmacokinetic parameters of Metformin- Ranolazine**
Show the Table 5: Pharmacokinetic parameters of Metformin (1000mg BID), Ranolazine (500mg BID) alone and in combination[12]. The drug-drug interaction between metformin and ranolazine was studied by Julia Zack and Jolene Berg. When metformin (1000mg BID) was co-administered with ranolazine (500mg BID), ranolazine increased the pharmacokinetic parameters such as metformin's $C_{max}$, $AUC_{tau}$, $t_{1/2}$, and CL/F, while metformin's $t_{max}$ remained unchanged when compared to metformin alone[12].

**Pharmacokinetic parameters of Furosemide-Tolvaptan**
Show the Table 6: Pharmacokinetic parameters of Furosemide, Tolvaptan alone and in combination[8].
The drug-drug interaction between tolvaptan and furosemide was investigated by researchers Susan E. Shoaf and Steven L. Bramer. Researchers found that when provided in combination with tolvaptan (30 mg), furosemide (80 mg) had lower pharmacokinetic characteristics such as $C_{max}$, $AUC_t$, $AUC_\infty$, %fe, u, and CLr, but increased $t_{1/2}$, CL/F but $t_{max}$ remained as unchanged when compared to furosemide alone. While CL/F, CLr, and $t_{max}$ did not alter when comparing Tolvaptan alone, pharmacokinetic variables such as $C_{max}$, $AUC_t$, $AUC_\infty$, $t_{1/2}$, %fe, u were all higher[8].

**Pharmacokinetic parameters of Furosemide in goat**

Show the Table 7: Pharmacokinetic parameter of Furosemide in goat with different routes likes IV, IM and SC routes[13]. #- values is statistically different than that in IV and IM administration ($p<0.05$) *- Values is statistically different than that in IM administration ($p<0.05$)

Gul Cetin, Orhan Corum and other researchers used IV, IM and SC injections to study the pharmacokinetics of furosemide in goates. Furosemide given by IV, IM and SC were found to have a no statistically significant variations between the administration route. The $AUC_{0-\infty}$ was higher with IV and IM treatment than with SC. After IV, the $CI_T$ and $V_{dss}$ were 0.30L/h/kg and 0.17L/kg respectively. Furosemide was administered by SC and IM, the corresponding $C_{max}$ values were 11.19 and 6.49µg/ml at 0.23 and 0.39hrs respectively. Following IM and SC dosage, the bioavailability was 70.80% and 109.84% respectively. The goat's $t_{1/2\lambda z}$, $V_{dss}$ and $CI_T$ were 0.71h, 0.17L/kg and 0.30L/h/kg after receiving IV furosemide treatment respectively[13].

**Pharmacokinetic parameters of Furosemide in hypertensive parturient women under caesarean section:**

Show the Table 8: Furosemide pharmacokinetic parameters in hypertensive parturient women receiving caesarean sections[14]. The maternal-fetal pharmacokinetic of furosemide were investigated by Paluo Vinicius, Fernanda and other researchers in hypertensive parturient women (n=12) undergoing caesarean delivery after a single oral dose of 40mg. The researchers observed that the median and interquartile range of the furosemide pharmacokinetic parameters, the following outcomes were obtained: $AUC_{0-12h}$-1366ng/ml, $AUC_{0-\infty}$-1580ng/ml, Vd/F-82.8L, CL/F-25.3L/h, $CL_R$-2.50L/h and $CL_{NR}$-22.7L/h. Furosemide concentration in 4 samples of amniotic fluid and 8 samples of umbilical cord were measured that shows a furosemide transplacental transfer of 11.0ng/ml and 45.8ng/ml respectively[14].

## CONCLUSION

From the table-3 we observed that, Metformin and Chiglitazar showed no clinically significant variations in the pharmacokinetics of the two drugs, even though the combination treatment resulted in a small reduction in CHI exposure. Furthermore, each group of subjects exhibited good tolerance. The impact of PGT on MET's AUC, $t_{max}$, $C_{max}$, $t_{1/2}$ and CL/F at a somewhat elevated concentration that is showed in table-4. PGT's role is an OCT inhibitor may have an impact on MET absorption and clearance. Consequently, to regulate plasma glucose, improve medication management, and prevent negative side effects, close patient observation and dose modification of the PGT and MET combination are advised. We noticed from Table-5 that, ranolazine co-administration raises metformin exposure most likely by ranolazine induced reduction of metformin renal excretion. At the lower ranolazine dose of 500mg BID, the dose-dependent increase in metformin exposure caused by ranolazine is not very significant. When metformin 1000mg BID and ranolazine 500mg BID were given together, the T2DM individuals tolerated the drugs well. Furosemide co-administration did not seem to have a clinically meaningful impact on the pharmacokinetics of tolvaptan as indicated in Table-6. When taken in combination with tolvaptan, the pharmacokinetics of furosemide did not seem to be considerably impacted. The aquaretic action of tolvaptan was not considerably impacted by furosemide. Plasma renin activity did not increase when tolvaptan was taken alone. At the prescribed dosage, tolvaptan was safe and well tolerated when used either alone or in conjunction with furosemide.

The delivery method had an impact on furosemide's plasma levels and bioavailability. As shown by the table-7, the duration of furosemide concentration above the minimum effective concentration (≥1µg/ml) for IV<IM and SC injection is similar, despite the observed variances making them appropriate routes for diuresis in goat. One possible way to reduce the risk of furosemide adverse effects related to the quick IV bolus injection is to use the IV route for a gradual injection or continuous infusion. When compared to nonpregnant healthy patients to hypertensive parturient women undergoing caesarean section appear to have a CL/F and non-renal $CL_{NR}$ of furosemide on the basis of table-8. Amniotic fluid concentrations are lower than those in the umbilical vein, and furosemide is transferred through the

placenta at a rate of about 40%. On a clinical basis, these findings imply that UGT isoenzyme substrates may have greater clearance during pregnancy and may need to have their dosage modified in this population.

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**Table 1: Pharmacokinetic analysis of metformin alone and in combination with other compounds using high-performance liquid chromatography (HPLC) with different species**.

| Drug/ Compounds | Chromatographic condition | Species | Dru-drug Interaction and Pharmacokinetic Study | Ref |
|---|---|---|---|---|
| Metformin - Chiglitazar (CHI) | RP-HPLC system coupling with tandem mass spectrometry having electrospray ionization. Mobile phase containing a mixture of acetonitrile and water containing 0.4% formic acid in a ratio of 95:5. | Both healthy male and female individuals with a BMI ranging from 19-26kg/m$^2$ who are least 18yrs of age. | self-controlled, three-period crossover, open-label, and randomized. In all, 15 subjects were divided into three groups. Group-I subject received MET 1000mg, CHI 48mg was received by group-II and the combination of MET (1000mg)+CHI(48mg) was received by group-III.<br>Blood samples were withdrawn at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36 and 48h, then the samples were centrifuged for 10min at 3500rpm.<br>pharmacokinetic parameters (PKs)were measured by using a mixed-effect model on log-transformed pharmacokinetic profile. | [11] |
| Metformin-Pioglitazone (PGT) | Agilent Eclipse Plus C18 column was maintained at room temperature of 25±2$^o$C. The combination of acetonitrile and 6mM ammonium formate with a pH of 4.5 with 0.1% formic acid made up the mobile phase. 0.5ml/min was the flow rate maintained. Triple-quadruple LC/tandem mass spectrometry was used for the study. | Male Wistar albino rats, 8wk old, weighing 200-220g. | Three groups of three rats each were selected by random selection. Rats in Group-I received MET (50mg/kg, orally(po)); Group-II received PGT (50mg/kg); Group-III received MET (50mg/kg, po) + PGT (50mg/kg, po).<br>After 0.5, 1, 2, 4, 6, 8, 12, and 24h, blood samples were collected and centrifuged for 10mins at 10000rpm.<br>From the experiment's data, $C_{max}$ and $t_{max}$ were determined. Regression analysis was used to estimate the Kel based on the slope and 0.693/Kel was utilized for calculating $t_{1/2}$. PKSolver was also used to compute the MRT, Vd/F and CL/F. | [3] |

| | | | | |
|---|---|---|---|---|
| Metformin-Ranolazine | LC-MS/MS system, a Phenomenex Synergi Hydro-R column (2x50mm) and the mobile phase was composed of acetonitrile and water with 0.1% formic acid with a pH of 3. | Both male and female patients, ages between 30-65, with a history of type 2 diabetes and BMI of 25–40 kg/m$^2$. | Open-labelled single cohort, multiple doses, 2-period fixed-sequence method. Participants were taken 2-fixed sequence treatment fasting 5days each: from days 1-5, metformin 1000mg BID (twice daily) and from days 6-10, metformin (1000mg, BID) and ranolazine (500mg, BID). Day 10, a single morning dose of 500 mg of ranolazine was given.<br>Samples were taken in the morning at 0.5, 1, 2, 4, 6, 8, 10, and 12h after the study medication was administered. Phoenix WinNonlin v6.2.1 was used to calculate the PKs parameter. | [12] |

**Table 2: Pharmacokinetic analysis of furosemide alone and in combination with other compounds using HPLC in various species to determine drug-drug interactions.**

| Drug/ Compound | Chromatographic condition | Species | Drug-drug interaction and Pharmacokinetic studies | Ref. |
|---|---|---|---|---|
| Furosemide-Tolvaptan | RP-HPLC system with UV-detector was used. | 12 white men, age from 18-29yr and with body weights of ranging 69-101kg, within 15% of the optimal body wt, were enrolled. | Three-period cross-over, single-centre randomized open-label parallel arm design. Six participants allocated to each of the two treatment groups. 30mg of tolvaptan, 80mg of furosemide and tolvaptan 30mg + furosemide 80mg were given to the subject Arm-1. 30mg of tolvaptan, 100mg of HCTZ and 30mg of tolvaptan + 100mg of HCTZ were given to the subjects in Arm-2. There was a 48h washout period between doses. Blood samples were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 16 and 24hrs. 5ml of blood were drawn in order to measure the tolvaptan and furosemide levels. Samples were centrifuged for 10min at 2500rpm. Both $C_{max}$ and $t_{max}$ were calculated from the data on plasma concentration-time | [8] |

| | | | | |
|---|---|---|---|---|
| | | | curve. WinNonlin Professional software was used for PKs calculations. | |
| Furosemide | HPLC-UV system with C18 column (250x4.6mm; 5µm, Phenomenex®). The temperature of the auto-sampler and column were kept at 24°C and 40°C respectively. Methanol (35%) and 0.01M sodium acetate (pH of 5.65%) were act as a mobile phase with a flow rate of 1ml/min. | Six female goats, 45 ± 5 kg in body weight and 2.4 ± 0.4 year old. | Three periods, crossover design, separated by a 15-day washout interval. The dosage of furosemide was 2.5mg/kg. Furosemide injections in single IV, IM and SC were administered to each goat, followed by a 15-days washout period. First, two goats received furosemide IV, two went IM, and two went SC.<br>Blood samples were drawn from each goat's right jugular vein at 0, 5, 10, 15, 20, 25, 30 and 45min as well as 1, 1.5, 2, 3, 4, 5 and 6h after the medicine was administered and centrifuging for 10min at 4000rpm. A non-compartmental technique was used to examine the furosemide concentrations in plasma using the WinNonlin 6.1.0.173 software application. | [13] |
| Furosemide | RP-HPLC system with fluorescence detector that carried out at 405 nm(emission) and 345 nm(excitation). C18 column (125 x 4mm, 5µm). The 80:20 mixture acetonitrile and 0.25M acetate buffer (pH 4.0) were used as the mobile phase. | 12 hypertensives parturient women undergoing cesarean sections were involved with gestational ages ranging from 36.0-40.4 wk. | Hypertensive parturient women who were on methyldopa (250mg/8hrs) and/or pindolol (10mg/12hrs) for the purpose of performing a cesarean section and obtaining biological samples. An IV catheter was used to draw 5ml of blood at 0, 0.5, 1, 2, 4, 6, 8 and 12 hrs following the administration of furosemide (40mg) through oral route prior to delivery. At birth, blood samples from the mother and the umbilical cord were also taken in order to evaluate the transplacental transfer. After centrifuging blood samples at 2000rpm for 15min. Up to 12h after the medicine was administered, | [14] |

| | | | | |
|---|---|---|---|---|
| | | | the entire volume of urine was collected at around 3h intervals. A sample of amniotic fluid was taken during the caesarean section. Phoenix WinNonlin, version 6.3, was used to assess the PKs profile of furosemide. | |

**Table 3: Pharmacokinetic parameters of Metformin, Chiglitazar alone and in combination[11].**

| Drugs/ Compounds | $C_{max}$ (ng/ml) | $t_{max}$ (h) | $t_{1/2}$ (h) | $AUC_{0-t}$ (ng*h/ml) | $AUC_{0-\infty}$ (ng*h/ml) | CL/F (L/h) |
|---|---|---|---|---|---|---|
| **Metformin alone** | 1790 | 3.0 | 8.09 | 12570 | 12710 | 80.42 |
| **Chiglitazar alone** | 1620 | 4.0 | 10.75 | 12540 | 12910 | 3.88 |
| **Metformin + Chiglitazar** | 1820 | 3.0 | 7.89 | 13190 | 13350 | 76.99 |
| | 1420 | 4.0 | 10.12 | 12130 | 12470 | 4.02 |

**Table 4: Pharmacokinetic parameters of Metformin, Pioglitazone alone and in combination[3].**

| Drugs/ Compounds | $C_{max}$ (ng/ml) | $t_{max}$ (h) | $AUC_{0-t}$ (ng*h/ml) | $AUC_{0-\infty}$ (ng*h/ml) | $t_{1/2}$ (h) | Kel (1/h) | Vd/F (L) | CL/F (L/h) |
|---|---|---|---|---|---|---|---|---|
| **Metformin alone** | 2310 ± 367 | 2.00 ± 0.43 | 10,830 ± 976 | 10,885 ± 876 | 2.84 ± 1.2 | 0.24 ± 0.134 | 1.7 ± 0.36 | 0.93 ± 0.26 |
| **Pioglitazone Alone** | 1920 ± 598 | 1.00 ± 0.3 | 18,214 ± 2189 | 22,967 ± 1762 | 10.81 ± 2.3 | 0.06 ± 0.02 | 5.5 ± 0.41 | 0.5 ± 0.2 |
| **Metformin + Pioglitazone** | 167 ± 88.2 | 0.50 ± 0.15 | 507 ± 158 | 563 ± 217 | 13.14 ± 2.1 | 0.05 ± 0.01 | 55.3 ± 0.88 | 20.5 ± 0.91 |
| | 64 ± 12.3 | 2.00 ± 0.75 | 207 ± 98 | 252 ± 101 | 16.68 ± 1.4 | 0.04 ± 0.01 | 70.5 ± 0.82 | 56.5 ± 0.01 |

**Table 5: Pharmacokinetic parameters of Metformin (1000mg BID), Ranolazine (500mg BID) alone and in combination[12].**

| Drugs/ Compounds | $C_{max}$ Mean (SD) | $AUC_{tau}$ Mean (SD) | $t_{max}$ Median (min, max) | $t_{1/2}$ Mean (SD) | CL/F, Mean (SD) |
|---|---|---|---|---|---|
| **Metformin 1000mg BID** | 2497 | 12933 | 1.5 | 4.0 | 83.2 |
| **Ranolazine 500mg BID** | 1180 | 8722 | 3.0 | 3.6 | - |
| **Metformin 1000mg BID + Ranolazine 500mg BID** | 3059 | 17920 | 1.5 | 4.4 | 60.8 |

**Table 6: Pharmacokinetic parameters of Furosemide, Tolvaptan alone and in combination[8].**

| Drug/ Compound | $C_{max}$ (ng/ml) | $t_{max}$ (h) | $AUC_t$ (µg*h/ml) | $t_{1/2,z}$ (h) | $AUC_\infty$ (µg*h/ml) | CL/F (ml/min/kg) | %fe,u | CLr (ml/min/ kg) |
|---|---|---|---|---|---|---|---|---|
| **Furosemide (80mg)** | 2.90 (1.00) | 1.5 (0.5–3) | 7.06 (1.62) | 2.6 (1.3) | 7.18 (1.64) | 2.52 (0.46) | 43.5 (10.3) | 1.12 (0.41) |
| **Tolvaptan** | 277 | 2 | 1.57 | 5.7 | 1.69 | 4.03 | 0.14 | 0.006 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **(30mg)** | (22) | (1.5–3) | (0.26) | (3.0) | (0.35) | (0.82) | (0.05) | (0.002) |
| **Furosemide (80mg)** | 2.61 (1.31) | 1.5 (1–4) | 6.40 (1.55) | 3.9 (2.5) | 6.55 (1.55) | 2.76 (0. 50) | 40.2 (11.8) | 1.09 (0.28) |
| **+ Tolvaptan (30mg)** | 327 (82) | 2 (1–4) | 1.94 (0.31) | 6.1 (3.1) | 2.08 (0.39) | 3.27 (0.75) | 0.15 (0.05) | 0.005 (0.002) |

**Table 7: Pharmacokinetic parameter of Furosemide in goat with different routes likes IV, IM and SC routes[13]. #- values is statistically different than that in IV and IM administration ($p<0.05$) *- Values is statistically different than that in IM administration ($p<0.05$)**

| parameters | SC | IV | IM |
|---|---|---|---|
| **$C_{max}$** | 6.49* | - | 11.19 |
| **$t_{max}$** | 0.39* | - | 0.23 |
| **$t_{1/2\lambda z}$ (h)** | 0.70 | 0.71 | 0.69 |
| **$AUC_{0-4}$ (μg*h/ml)** | 5.88# | 8.38 | 9.16 |
| **$AUC_{0-\infty}$ (μg*h/ml)** | 5.99# | 8.46 | 9.29 |
| **$AUC_{extrap}$ (%)** | 1.81 | 0.93 | 1.37 |
| **MRT (h)** | 0.45* | - | 0.30 |
| **$CI_T$ (L/h/kg)** | - | 0.30 | - |
| **$V_{dss}$ (L/kg)** | - | 0.17 | - |

**Table 8: Furosemide pharmacokinetic parameters in hypertensive parturient women receiving caesarean sections[14].**

| Parameter | Values |
|---|---|
| **$C_{max}$ (ng/ml)** | 403 |
| **$t_{max}$ (h)** | 2.00 |
| **$AUC_{0-12h}$ (ng*h/ml)** | 1366 |
| **$AUC_{0-\infty}$ (ng*h/ml)** | 1580 |
| **$t_{1/2}$ (h)** | 2.50 |
| **Kel (1/h)** | 0.27 |
| **Vd/F (L)** | 82.8 |
| **CL/F (L/h)** | 25.3 |
| **$CL_{NR}$ (L/h)** | 22.7 |
| **$CL_R$ (L/h)** | 2.50 |
| **Time between drug administration and delivery (h)** | 2.50 |
| **Umbilical vein, n=8 (ng/ml)** | 45.8 |
| **Amniotic fluid, n=4 (ng/ml)** | 11.0 |
| **Maternal plasma, n=8 (ng/ml)** | 140 |
| **Umbilical vein/maternal plasma ratio (n=8)** | 0.43 |

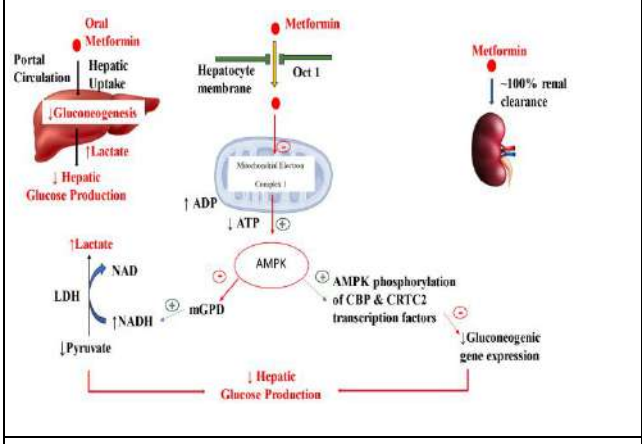


**Figure 1: Mechanism of action metformin.**

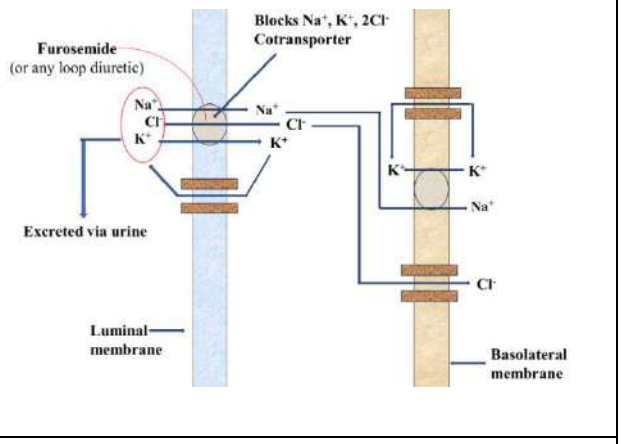


**Figure 2: Mechanism of action of Furosemide.**

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**RESEARCH ARTICLE**

# Physico – Chemical and Phytochemical Analysis of Siddha Polyherbal Formulation – Megarajanga Kirutham

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## ABSTRACT

Standardization is the main part of drug developing process to determine the quality of medicine. The quality of Siddha formulations must be determined before it is used in preclinical and clinical studies. Megarajanga Kirutham (MRK) is the polyherbal Siddha formulation indicated for menorrhagia, dysuria, leucorrhoea, anemia and emaciation. It was processed as per the procedure mentioned in classical Siddha text "Sigicha rathna deepam". To understand the quality and changes that occurred during the preparation, Megarajanga Ghritam was analyzed by using modern techniques. After the analysis, it was found that the pH, acid value, Saponification value and the Iodine value were 6, 1.5, 205 and 101 respectively. Phytochemical screening affirmed the presence of flavonoids, steroids, sugars, triterpenoids, coumarins and phenols in the extract. This preliminary study helped in authenticating and ensuring the quality of Megarajanga Kirutham.

**Keywords:** Megarajanga Kirutham, standardization, physico chemical, saponification value, iodine value, Siddha.

## INTRODUCTION

The Siddha medical system is an ancient medical science that helps people by treating a variety of chronic illnesses [1]. Herbs, metals, minerals, and other materials have long been used by Siddha medicine to create its medical concoctions [2]. Traditional wisdom believes that herbal treatments are safe, and an increasing number of people are utilizing them worldwide [3]. However, there are still no appropriate standards techniques for determining its effectiveness, consistency, and quality [4]. The importance of qualitative and quantitative methods for sample characterization, biomarker and/or chemical marker detection, and fingerprint profile analysis is emphasized by the World Health Organization (WHO) [5]. Nowadays, individuals also require further scientific validation about the pharmacokinetics, safety, etc., of conventional medications [6]. Drug evaluation is carried out to confirm the substance's identity, confirm its quality and purity, and detect any instances of adulteration [7].

A parameter that represents the purity and quality of a material is called a standard. Standardization refers to the procedure of creating a norm for a certain drug [8]. In the Siddha medical system, Megarajanga Kirutham is a well-known polyherbal formulation that is frequently used for many health conditions, according to the Siddha canonical literature "*Sigicha Rathna Deepam*". It has the indication for menorrhagia, dysuria, leucorrhoea, anemia and emaciation [9]. Herbal medicines are in increasing demand for primary healthcare worldwide due to their increased safety margins and cost effectiveness [10]. However, as of now, there is lack of analytical standards for Meharajanga Kirutham. Hence, this formulation was evaluated for physico-chemical and phytochemical analysis in order to establish specific quality requirements for the formulation.

## MATERIALS AND METHODS

**Collection of raw materials**

All of the raw drugs needed to prepare the test drug were bought from a reputable raw drug seller, Tambaram and the Medicinal botanist in National Institute of Siddha, Chennai, provided their authentication. After appropriate purification, the trial medication was manufactured in the Gunapadam Lab, National Institute of Siddha, Chennai.

**Purification of raw drugs:**

All the herbal drugs are purified as per the classical Siddha text Gunapadam Mooligai Vaguppu [11].

**Method of preparation**

The ingredients of megarajanga Kiruthamwas mentioned in the Table 1. The drug was prepared as per the Siddha classical text "Sigicha rathna deepam[9]". 350grams of aththi pattai (*Ficus racemosa*), Naaval pattai (*Syzygium cumini*), othiyampattai (*Lannea coromandelica*) were crushed and to prepare the kudineer (decoction) 8 liters of water was added and reduced to 1 liter. Then juices of karumbu juice (*Saccharum officinarum),* nellikai juice (*Emblica officinalis*), lemon juice (*Citrus limon*), gingelly oil (*Sesamum indicum*) and cow's ghee were added into the decoction and boiled well. Each 26.25 grams powders of thalisapathiri (*Abies spectabilis*), elam (*Elettaria cardamomum*), kirambu*(Syzygium aromaticum),* jadhikkai (*Myristica fragrans*), chitrarathai (*Alpinia officinarum*), athimathuram (*Glycyrrhiza glabra*) and nilappanai kizhangu (*Curculigo orchioides*) were ground with the milk of three coconuts (*Cocos nucifera*). This *kalkam* was added to the decoction mixture and boiled until the ghee consistency.

**Quality evaluation of Megarajanga Kirutham**

The formulation was initially analyzed for organoleptic parameters like color, odor, taste, texture, etc. Various physico-chemical parameters which are specific for Kiruthampreparations such as loss on drying, pH value, refractive index, acid value, saponification value, iodine value and rancidity were evaluated [12,13].

**Percentage loss on drying**

10gm of test drug (weight equivalent to oil) was accurately weighed in evaporating dish. The sample was dried at 105ºC for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

**Determination of Total Ash**

3 gm of test drug (weight equivalent to oil) was accurately weighed in silica dish and incinerated at the furnace a temperature 400ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

**Determination of pH**

Megarajanga Kirutham being oily in nature the direct litmus evaluation method was adopted to check the pH of the sample.

**Determination of Iodine value**

About 20 gm of oil was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 minutes and refrigerated for an hour. About 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow color. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue color indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.

**Determination of saponification value**

About 2 gm (weight equivalent to oil) of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeat the same procedure without taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

**Rancidity Test**

1 ml of melted fat was mixed with 1ml of conc. HCl and 1 ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

**Acid Value**

2-10gm of Kirutham weighed in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralized with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink color. The experiment repeated twice to get concordant values.

**Phytochemical analysis of formulation:**

Megarajanga Kirutham (MRK) was extracted with hexane and the extract was subjected to the following analysis [14].

**Test for Alkaloid- Mayer's reagent**

To the test drug about 2ml of Mayer's reagent was added and was observed for the presence of alkaloids. Appearance of dull white precipitate indicates the presence of alkaloids.

**Test for flavonoid**
To 0.1ml of the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

**Test for Glycosides -Borntrager's Test**
MRK is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink color indicates presence of glycosides.

**Test for Triterpenoids**
To the test solution 2ml chloroform was added with few drops of concentrated Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

**Test for Steroids - Salkowski test**
To the test solution 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

**Test for Carbohydrates - Benedict's test**
To 0.5 ml of test drug about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

**Test for Phenol- Lead acetate test:**
The test sample is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

**Test for tannins**
About 0.5ml of test sample is boiled in 20ml of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. The 0.1% $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins

**Test for Saponins**
The test drugs were shaken with water vigorously for 10 minutes, copious lather formation indicates the presence of saponins.

**Test for Proteins (Biuret Test)**:
Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

**Test of Coumarins**
1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

**Test for Anthocyanin**
About 0.2 ml of the extract was weighed in separate test tube, 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at 100 ± 2°C. The appearance of bluish green color indicates the presence of anthocyanin.

**Determination of total Phenol content**
The total phenol content was determined using Folin–Ciocalteu reagents with analytical grade Gallic acid as the standard. 1 ml of sample was added to deionized water (10 ml) and Folin–Ciocalteu phenol reagents (1ml). After 5

minutes, 20% sodium carbonate (2 ml) was added to the mixture. After being kept in total darkness for 1 hr, the absorbance was measured at 750 nm using a spectrophotometer. Amounts of total Phenol was calculated using Gallic acid calibration curve. The results were expressed as Gallic acid equivalents (GAE) mg/g of dry plant matter [15].

**Total Flavonoid**

Total flavonoid content in the drug MRK was determined using aluminum chloride method. In this method, Quercetin was used as standard and flavonoid contents were measured as quercetin equivalent. For this purpose, the calibration curve of quercetin was drawn. 1ml of standard or sample MRK was taken into 10ml volumetric flask, containing 4ml of distilled water. 0.3ml of 5% $NaNO_2$ added to the flask. After 5min, 0.3ml 10% AlCl3 was added to the mixture. At the 6th minute add 2ml of 1M NaOH was added and volume made up to 10ml with distills water. The absorbance was noted at 510nm using UV-Visible spectrophotometer [16].

## RESULTS AND DISCUSSION

The cornerstone of Siddha medicine, which is utilized to treat illnesses and maintain health, is herbal remedies and formulations[17]. Many Siddha formulations are produced and marketed by pharmaceutical companies in accordance with regulatory guidelines and classical texts. However, on a cursory glance, significant differences can be observed between the identical formulations produced by various companies. This raises questions about the quality standards [18]. In the present study, Megarajanga Kirutham was standardized using the modern scientific techniques. The organoleptic analysis of MRK revealed that it was yellowish liquid with characteristic odor and greasy in nature mentioned in Table 2.

Physico chemical characteristics such as rancidity, acid value, saponification value, pH, refractive index, and iodine value were assessed and mentioned in Table 3. The MRK pH value of 6 indicates a somewhat acidic environment [19]. It might occur as a result of the acidic ingredients of this formulation like amla juice and lemon juice [20,21]. At 25ºC, the test drug's Refractive Index value is 1.48. It provides information about a substance's concentration, purity, and viscosity [22]. The type of fatty acid that is present in the fat determines the saponification value. The saponification value of MRK is 205. The presence of long chain fatty acids in the formulation is indicated by the saponification value [23,24]. Unsaturated free fatty acids are indicated by a high value. Compared to saturated fatty acids, unsaturated fatty acids are more readily absorbed. The iodine value of MRK was discovered as 101 [25]. The presence of free fatty acids in the oil, which cause compounds to get rancid, is indicated by the acid value. The acid value of MRK is 1.5. Less acid value indicates the less chance of decomposition [26]. Total ash value of MRK is 0.788. The amount of ash in a crude drug also indicates the level of purity in crude and the produced drug, as well as the care used in drug preservation [27]. The obtained values of the test drug were found in normal limits which indicate the good quality of product. Rancidity also not found in this formulation.

Phytochemical constituent of the herbal drug is responsible for the therapeutic effect of the formulation. So, evaluation of phytochemical constituents in this test drug is the needed one. Pytochemical screening of MRK showed in Table 4. This study revealed the presence of flavonoids, steroids, sugars, triterpenoids, coumarins and phenols. These compounds are responsible for the pharmacological activity of this formulation. Triterpenoids derived from the licorice possess the anti bacterial and anti fungal properties [28]. Antibacterial and antioxidant activity present in *Ficus racemosa* and *Syzygium cumini* responsible in the treatment of leucorrhoea [29,30]. The Phenolic and flavonoid compounds are regarded as the most significant types of phytochemicals because of their various biological characteristics. The total phenol and flavonoid contents of MRK is 0.934 ± 0.04gae mg/gm and 2.82 ± 0.42quercetin mg/gm. Recently phenolics have been considered powerful antioxidants in vitro and proved to be more potent than vitamin C and E [31].

## CONCLUSION

The outcomes of standardization techniques including phytochemical, microbiological, chromatographic, and heavy metal analyses can be utilized as reference standards for other pharmaceutical procedures. It could also be a useful source of data for further studies to determine the formulation and evaluate its purity and quality. Further preclinical and clinical research will be required in the future to investigate additional parameters pertaining to the therapeutic effect of Megarajanga Kirutham.

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**CONFLICT OF INTEREST**
Declared none

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**Table 1: Ingredients of Megarajanga Kirutham**

| S. No | Tamil name | Botanical name | Quantity |
|---|---|---|---|
| 1. | Aththi pattai | *Ficus racemosa* Linn | ¼ Veesai (350 grams) |
| 2. | Naaval pattai | *Syzygium cumini* Linn | ¼ Veesai (350 grams) |
| 3. | Othiyampattai | *Lannea coromandelica* (Houtt)Merr | ¼ Veesai (350 grams) |
| 4. | Karumbu juice | *Saccharum officinarum* Linn | ½ padi (670ml) |
| 5. | Nellikai juice | *Phyllanthus emblica* Linn | ½ padi (670ml) |
| 6. | Lemon juice | *Citrus limon* Linn | ½ padi (670ml) |
| 7. | Gingelly oil | *Sesamum indicum* Linn | ½ padi (670ml) |
| 8. | Cow's ghee | | ½ padi (670ml) |
| 9. | Kirambu | *Syzygium aromaticum* Linn | ¾ palam (26gms) |
| 10. | Thalisapathiri | *Abies spectabilis* (D.Don)Mirb | ¾ palam (26gms) |
| 11. | Elam | *Elettaria cardamomum* Linn | ¾ palam (26gms) |
| 12. | Jadhikkai | *Myristica fragrans* Houtt | ¾ palam (26gms) |
| 13. | Chitrarathai | *Alpinia officinarum* Hance | ¾ palam (26gms) |

| | | | |
|---|---|---|---|
| 14. | Athimathuram | *Glycyrrhiza glabra* Linn | ¾ palam (26gms) |
| 15. | Nilappanai kizhangu | *Curculigo orchioides* Gaertn | ¾ palam (26gms) |
| 16. | Coconut milk | *Cocos nucifera* Linn | |

**Table 2: Organoleptic characters of Megarajanga Kirutham**

| Parameter | Observation |
|---|---|
| Color | Yellowish |
| Smell | Characteristic |
| Touch | Greasy |
| Appearance | Turbid |

**Table 3: Physico – chemical analysis of Megarajanga Kirutham**

| S.No | Parameter | MRK |
|---|---|---|
| 1 | Loss on Drying at 105 °C (%) | 8.03 |
| 2 | Total Ash (%) | 0.788 |
| 3 | pH | 6 |
| 4 | Refractive index | 1.48 |
| 5 | Iodine value (mg I2/g) | 101 |
| 6 | Acid value | 1.5 |
| 7 | Rancidity | Not oxidized |
| 8 | Saponification Value (mg of KOH to saponify 1gm of fat) | 205 |

**Table 4: Phytochemical screening of Megarajanga Kirutham**

| Phytocomponents | MRK |
|---|---|
| Alkaloids | - |
| Flavonoids | + |
| Glycosides | - |
| Steroids | + |
| Sugar | + |
| Triterepnoids | + |
| Coumarins | + |
| Phenols | + |
| Tannins | - |
| Saponins | - |
| Proteins | + |
| Anthocyanin | - |
| Total phenols (gae mg/gm) | 0.934 ± 0.04 |
| Total flavanoids (quercetin mg/gm) | 2.82 ± 0.42 |

+ indicates positive; - indicates negative

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**RESEARCH ARTICLE**

# A Study on an Irregular Colouring of Sunlet and Pangraph Families

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**ABSTRACT**

An irregular coloring is a proper coloring in which distinct vertices have different color codes. In this paper we obtain the irregular coloring of Sunlet and Pangraph families are studied.

**Keywords:** Central graph, Graph coloring, Irregular chromatic number, Irregular coloring ,Middle graph, Pan graph , Sunlet graph and Total graph

## INTRODUCTION

Let G be a finite, undirected graph with no loops and multiple edges. The graph G has the vertex set V (G) and the edge set E (G).Graph coloring is coloring of G such that no two adjacent vertices share the same color.
A proper coloring of a graph is an **irregular coloring**,if no two like-colored vertices have the same color code . i.e.,for every pair of vertices u and w ,code (u)≠code (w) whenever c(u)=c(w). Thus an Irregular coloring distinguishes each vertex from each of other vertex by its color or by its color code.
The **central graph** C (G) of a graph G is obtained by subdividing each edge of G by exactly once and joining all the non adjacent vertices of G.
The **middle graph** of G denoted by M(G), is defined as follows :
The vertex set of M(G) is V(G)∪E(G) in which two elements are adjacent in M(G) if the following conditions hold.(i) x,y∈E(G) and x, y are adjacent in G.(ii) x ∈V(G), y ∈ E(G) and y is incident on x in G.
The total graph T(G) of a graph G is a graph such that the vertex set of T corresponds to the vertices and edges of G and two vertices are adjacent in T iff their corresponding elements are either adjacent or incident in G.

**Irregular Coloring Of Sunlet Graph Families**

**Theorem 2.1 :** For the central graph of Sunlet graph ,the irregular chromatic number is n+2
(i.e) $\chi_{ir}[C(S_n)] = n+2, n\geq 3$

**Proof.**

Let G = C (Sn )be the central graph of sunlet graph.
Let $v_1, v_2 \ldots v_n$be the inner vertices of the cycle graph $C_n$
Let $u_1, u_2, \cdots u_n$ be the pendant vertices .
The inner sub-division of the cycle graph $C_n$ are$v_{i,i+1}, v_{i+1,i+2}, \cdots v_n, v_1$
The outer subdivision pendant vertices are $f_1, f_2, \ldots f_n$
Now the coloring assignment of C(Sn) is as follows.
The inner vertices $v_1, v_2 \ldots\ldots\ldots v_n$ of the cycle graph $C_n$and the outer pendant vertices $u_1, u_2 \cdots u_n$ are colored are colored with the colors $c_1, c_2, c_3, \cdots c_n$
The inner sub-division vertices $v_{i,i+1}, v_{i+1,i+2} \ldots\ldots v_n, v_1$of the cycle graph $C_n$ are colored with the colors $c_3, c_4, \cdots c_{n+2}$
The outer sub-division pendant vertices $f_1, f_2, \cdots f_n$ are colored with the color $c_{n+1}$
since $deg(u_i) \neq deg(v_i)$,it shows that $code(u_i) \neq code(v_i)$
Hence an irregular chromatic number of central graph of sunlet graph is 2n+3.
(i.e)$\chi_{ir}[[C(S_n)] = +2$ , n≥3

**Theorem 2.2 :** For the middle graph of Sunlet graph ,the irregular chromatic number is 2n+3 (i.e) $\chi_{ir}[M(S_n)] = 2n+3, n\geq 3$

**Proof.**

Let G = M (Sn )be the middle graph of sunlet graph.
Let $v_1, v_2 \cdots, v_n$be the inner vertices of the cycle graph $C_n$and $u_1, u_2, \cdots u_n$ be the pendant vertices . The inner sub-division of the cycle graph $C_n$ are$v_{i,i+1}, v_{i+1,i+2}, \cdots v_n, v_1$ and the outer subdivision pendant vertices are $f_1, f_2, \ldots f_n$
Now the coloring assignment of M(Sn) is as follows.
The inner vertices $v_1, v_2 \ldots\ldots\ldots v_n$ of the cycle graph $C_n$ are colored with the colors $c_1, c_2, c_3, \cdots c_n$
The inner sub-division vertices $v_{i,i+1}, v_{i+1,i+2} \ldots\ldots v_n, v_1$of the cycle graph $C_n$ are colored with the colors $c_3, c_4, \cdots c_{n+2}$
The outer pendant vertices $u_1, u_2 \cdots u_n$ are colored with the color$c_{n+3}$
The outer sub-division pendant vertices $f_1, f_2, \cdots f_n$ are colored with the color $c_{n+4}, c_{n+5}, \cdots c_{2n+3}$
since $deg(u_i) \neq deg(f_i)$,it shows that $code(u_i) \neq code(f_i)$
Since each $u_i$'s are adjacent to $f_i$'s but $u_i$'s are not adjacent to $v_i$'s.
It follows that, code $(u_i) \neq code(v_i)$.
Hence an irregular chromatic number of middle graph of sunlet graph is 2n+3.
(i.e)$\chi_{ir}[[M(S_n)] = 2n+3$ , n≥2

**Theorem 2.3 :** For the total graph of Sunlet graph ,the irregular chromatic number is 2n+3(i.e) $\chi_{ir}[T(S_n)] = 2n+3, n\geq 3$

**Proof.**

Let G = T ($S_n$ )be the middle graph of Sunlet graph.
Now the coloring assignment ofT(Sn) is as follows.
The inner vertices $v_1, v_2 \ldots\ldots\ldots v_n$ of the cycle graph $C_n$ are colored with the colors $c_1, c_2, c_3, \cdots c_n$
The inner sub-division vertices $v_{i,i+1}, v_{i+1,i+2} \ldots\ldots v_n, v_1$of the cycle graph $C_n$ are colored with the colors $c_3, c_4, \cdots c_{n+2}$
The outer pendant vertices $u_1, u_2 \cdots u_n$ are colored with the colors$c_{n+4}, c_{n+5}, \cdots c_{2n+3}$
The outer sub-division pendant vertices $f_1, f_2, \cdots f_n$ are colored with the color$c_{n+3}$
To prove (2n+3)- coloring is an irregular coloring of $T(S_n)$,
since$deg(u_i) \neq deg(f_i)$,it shows that $code(u_i) \neq code(f_i)$
Since each $u_i$'s are adjacent to $f_i$'s but $u_i$'s are not adjacent to $v_i$'s.
It follows that, code $(u_i) \neq code(v_i)$.
Hence anirregular chromatic number of total graph of sunlet graph is 2n+3.
(i.e)$\chi_{ir}[[T(S_n)] = 2n+3$ , n≥2

**Remark:**
For central ,middle and total graph of sunletgaph, we can give same colors in different manner.

**Irregular Coloring of Pan Graph Families**
**Theorem 3.1:** For the central and total graph of Pan graph,the irregular chromatic number is n+2. (i.e)$\chi_{ir}[C(P_n)] = \chi_{ir}[M(P_n)] = \chi_{ir}[T(P_n)]=n+2$
**Proof:**
The n-pan graph is the graph obtained by joining a cycle graph $C_n$ to a singleton graph$K_1$ with a bridge. The n-pan graph is isomorphic with the (n,1) tadpole graph.
Let $G=C(P_n)$ be the central graph of pan graph.
Now the coloring assignment is as follows.
**Case(i):For central graph of pan graph**
The outer vertices$\{v_1, v_2,....v_n\}$of the cycle graph are colored with the colors $\{c_1, c_2, c_3, …….c_n\}$ (clockwise)
The outer- intermediate vertices$\{ u_1, u_2,....u_n\}$ are colored with the colors $\{ c_3,c_4,…….c_{n+2}\}$ (clockwise)
The outer- intermediate vertex $w_1$ is colored with the color $c_1$
At last, the vertex of the singleton graph $k_1$ is colored with the color $c_2$
**Case(ii):For middle graph of pan graph**
The proof for case( ii) is similar as in case(i)
**Case(ii):For total graph of pan graph**
The outer vertices$\{v_1, v_2,....v_n\}$of the cycle graph are colored with the colors $\{c_1, c_2, c_3, …….c_n\}$ (clockwise)
The outer- intermediate vertices$\{ u_1, u_2,....u_n\}$ are colored with the colors $\{ c_3,c_4,…….c_{n+2}\}$ (clockwise)
The outer- intermediate vertex $w_1$ is colored with the color $c_1$
Atlast, the vertex of the singleton graph $k_1$ is colored with the color $c_{n+2}$
**From cases (i),(ii) and (iii)**
sincedeg($u_i$)≠deg($v_i$),it shows that code($u_i$)≠code ($v_i$)
Since each $u_i$'s are adjacent to $v_i$'s but $u_i$'s are not adjacent to $w_i$'s.
It follows that, code ($u_i$)≠code($w_i$).
Hence an irregular chromatic number of total graph of Pan graph is n+2.
$\chi_{ir}[C(P_n)]= \chi_{ir}[M(P_n)] = \chi_{ir}[T(P_n)]=n+2$

**Remark:**
For central ,middle and total graph of pan graph, we can give same colors in different manner.

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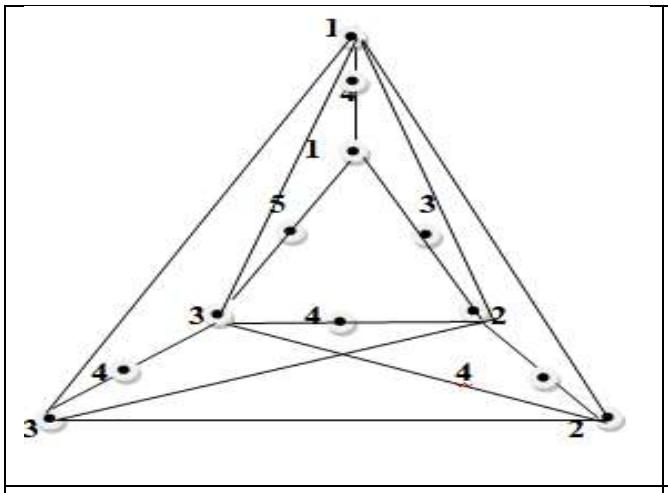


**Fig 1: Irregular coloring of C($S_3$)**

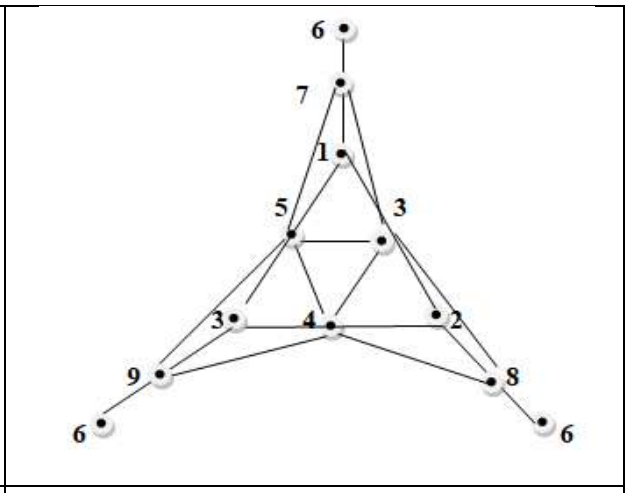


**Fig 2 : Irregular coloring of M($S_3$)**

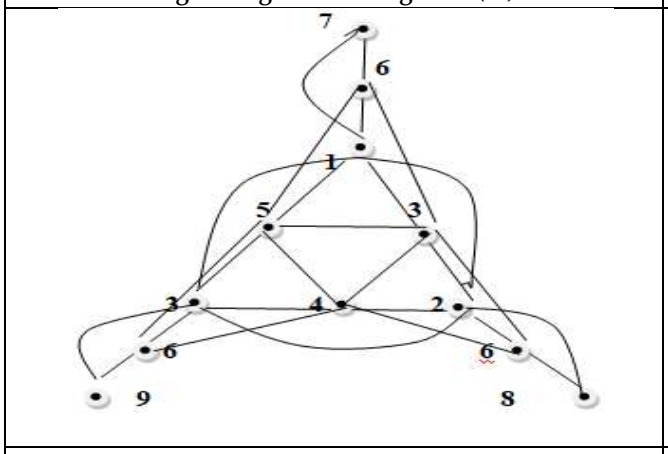


**Fig 3: Irregular coloring of T($S_3$)**

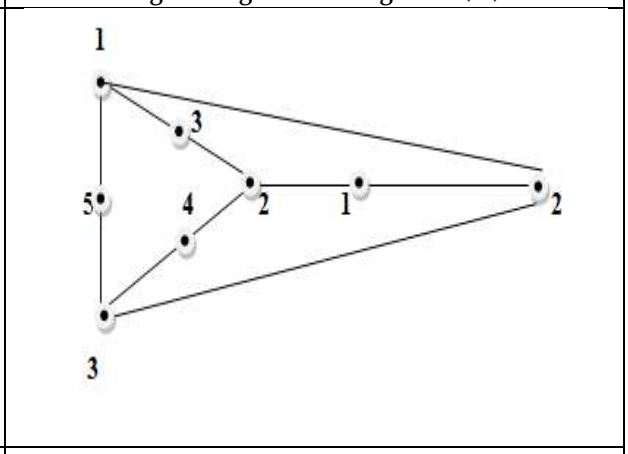


**Fig 4. Central graph of Pan graph $P_3$**

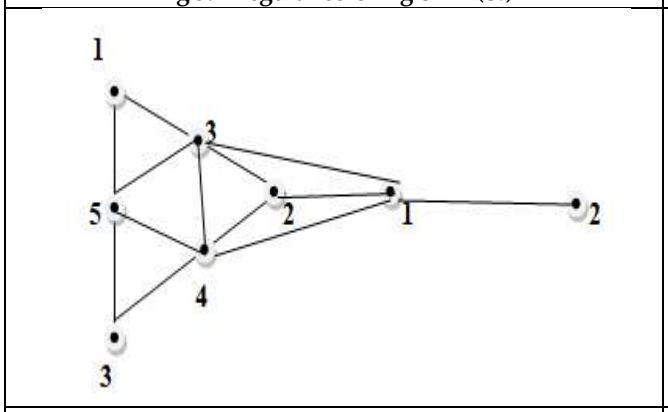


**Fig 5. Middle graph of Pan graph $P_3$**

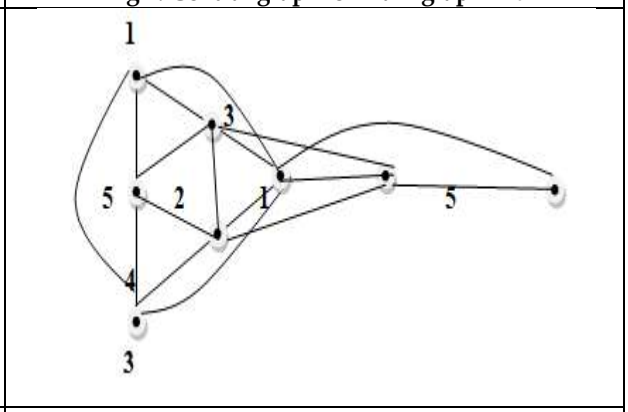


**Fig 6. Total graph of Pan graph $P_3$**

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**RESEARCH ARTICLE**

# A Comprehensive Analysis of Glaucoma Detection using Machine Learning and Deep Learning : A Survey

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**ABSTRACT**

Glaucoma is a degenerative eye disease that, if unchecked, causes permanent blindness. Efficient illness management requires prompt diagnosis and prompt treatment. Ophthalmologists have found some success in using ML and DL methods to aid in the diagnosis of glaucoma in recent years. The present state-of-the-art in glaucoma detection using ML and DL-based techniques is thoroughly examined in this review. To set the stage, we will go over the basics of glaucoma's pathogenesis and the eye's anatomy. After that, we explore the several ML and DL methods used for glaucoma detection, such as SVMs, RNs, and convolutional and recurrent neural networks. This article delves into the possibilities of computer-assisted techniques for glaucoma illness and staging, reviewing 30 research articles on the topic. Important parameters for automated diagnosis are examined in this study, including optic disc shape, cup-to-disc ratio, and retinal nerve fiber layer thickness. Furthermore, we go over some of the difficulties encountered when trying to create reliable ML/DL models for glaucoma detection, including issues with data scarcity, class imbalance, and the interpretability of model conclusions.

**Keywords:** Convolutional Neural Networks, Early detection, Glaucoma, Machine learning, deep learning

## INTRODUCTION

Without prompt diagnosis and treatment, glaucoma, a degenerative eye condition, may cause permanent damage to the optic nerve and eventually cause blindness. A primary cause of blindness, it affects millions of people globally [1-4]. Preserving eyesight and improving patient outcomes are paramount when it comes to timely identification and management. However, glaucoma may be difficult to diagnose and often calls for specialist tools and knowledge. Clinical glaucoma diagnosis has been greatly improved in recent years because to the incorporation of deep learning

(DL) and machine learning (ML) into medical imaging [5–11]. These innovations use computer algorithms to sift through mountains of data, such retinal scans, in search of traits and patterns that could indicate glaucomatous alterations. The glaucoma screening, diagnostic, and monitoring processes might be completely transformed by this paradigm shift towards automated or semi-automatic detection technologies [12–14].

The purpose of this review is to provide a thorough evaluation of the present state of glaucoma detection methods based on ML and DL. To provide the groundwork for a good grasp, we shall investigate the pathophysiology and anatomy of glaucoma [15–17]. After that, we will go over the merits, weaknesses, and performance measures of the different ML and DL algorithms used for glaucoma detection [18–20]. We will also talk about the benefits and drawbacks of using publically accessible datasets to train and test glaucoma detection algorithms. We will also discuss the main obstacles, such a lack of data, an imbalance in the classes, and the difficulty in understanding the reasoning behind the model's conclusions, that have been identified as preventing the development of reliable and clinically relevant ML/DL models for the identification of glaucoma [21–27]. Our goal is to discover current trends, research gaps, and future prospects in ML/DL-based glaucoma detection by reviewing and combining the current literature and advances in this area. We want to contribute to the development of scalable solutions that are reliable, accurate, and can be smoothly incorporated into clinical practice [28-31].

**Background study**

Afreen, N., & Aluvalu, R. (2024) a number of glaucoma detection techniques were examined in depth in this article. Since glaucoma was one of the most deadly eye diseases that may cause blindness without any noticeable symptoms, it was critical to diagnose the disease early on so that patients can prevent irreversible vision loss. Research has shown that deep learning models may aid in the early detection process. AlShawabkeh, M. et al. (2024) there was great potential for improving early detection, accuracy, and accessibility in glaucoma diagnoses via the application of AI. A number of models have shown promise as sensitive screening tools for glaucoma, but it was still difficult to create diagnostic models that can be used in specialized glaucoma care clinics by combining multi-modal anatomical and functional datasets. Bragança, C. P.et al. (2024) there has been tremendous success in distinguishing between glaucomatous and non-glaucomatous digital fundoscopic pictures, thanks to the invention of glaucoma classification algorithms. Nevertheless, at the present time, no software was accessible for actual clinical applications, even though many research have shown that AI algorithms may be used to aid in the diagnosis of glaucoma.

Hasan, M. et al. (2024) these authors research systematically analyzed the high-quality literature on AI-based glaucoma and neurodegenerative disease diagnostics published in the last 10 years. Analysis of the included research reveals that AI significantly improves glaucoma and neurodegenerative disease diagnoses compared to human doctors (area under the curve: 0.71-0.98, accuracy: 71.5%, area under the curve: 0.86). Jain, A., & Sakalle, V. (2024) Recent advances in medical diagnostics using ML and DL have created revolutionary new opportunities, particularly in the field of ophthalmology. The author highlight the tremendous potential of ML and DL models for glaucoma optic disk localization and classification in these authors assessment of these approaches to improve early identification and treatment of the disease.

L. Li et al. (2020) As a novel deep learning approach for automated glaucoma identification and abnormal region localization on fundus pictures, the author present AG-CNN in this research. Therefore, glaucoma might be identified by using the in-depth characteristics emphasized by the visual maps of diseased regions, which were derived from the anticipated attention maps. Shyamalee, T. et al. (2024) these authors research presented a deep learning-based method that incorporates segmentation, classification, and explainability to identify glaucoma illnesses using different fundus image datasets. The author demonstrated the system's usability and the veracity of the results after deploying the suggested method as a web application. Using explainable methodologies, this study aimed to identify the reasons behind the underlying classification model's prediction. Because of this, people were more likely to have faith in the system and be comfortable using it to aid in medical diagnosis. Subbarayudu, Y. et al. (2024) on the test data, the Logistic Regression model reached an accuracy of almost 95%. When compared to other models, including SVM, Random Forest, Decision Tree, and Naive Bayes, it achieved greater accuracy. A larger

number of false negatives (12) than false positives (5) was shown by the confusion matrix, suggesting that the model had a harder time accurately detecting class 1 photos with glaucoma than class 0 images without the condition. As a measure of the model's accuracy in classifying positive occurrences (glaucoma) out of all instances predicted as positive, the precision was determined to be 0.84. The accuracy in classifying positive events out of all the real positive instances was determined to be 0.94, which was known as the recall. Tang, Q. Y. et al. (2024) the aberrant WM functional abnormalities seen in PACG patients in this research mostly affect areas of the brain that were involved in vision. From a different WM functional angle, these authors results provide light on extensive brain damage in PACG. In addition, the aberrant ALFF values in brain WM that have been reported may be a neurological marker that may differentiate PACG patients from healthy controls.

M. Aloudat and M. Faezipour (2016) A increased risk of glaucoma was associated with a high proportion of red sclera in the excised tissue, according to these investigators' approach. M. N. Bajwa et al. (2016) Historically, glaucoma diagnostic Retinal Fundus photos (RFI) datasets have been rather tiny, consisting of just a few hundred photos, and have been meticulously acquired in controlled laboratory settings. None of the photos in these datasets include extra-retinal artifacts since the creators didn't care about the limits of imaging technology. There were a lot of eye disorders, according to the research, thus deep learning systems trained on these datasets don't do well. N. A.Diptu et al. (2018) the method developed by these writers was useful for the automated diagnosis of glaucoma in fundus images. This authors' technique successfully identifies the intermediate stage of glaucoma, which was a crucial component of any screening system for the condition. The region-of-interest (ROI) segmentation was used to detect bleeding in a specific area, which allowed for the automated identification of glaucoma issues. Due to its emphasis on localizing hemorrhage sites, the authors' technique proved computationally efficient. Applying this authors' method yielded notable results.

R. Panda et al. (2017) the author suggested a novel method for identifying RNFLDs to aid ophthalmologists in their endeavors to screen big populations for glaucoma. Accurately identifying the real border pixels may be achieved by feeding the RNN training data with freshly produced patch attributes. R. Zhao et al. (2020) Instead of using the segmentation phase that was often employed in traditional approaches, a methodology was devised to immediately estimate the Cup-to-Disc Ratio (CDR) value from fundus photographs. By combining semi-supervised learning with random regression forest, the author can make pictures more expressive and find the optimal CDRs to represent different image qualities. Following evaluation on a challenging glaucoma dataset, the authors' direct approach accurately calculated the CDR value, demonstrating strong correlations to values recorded manually. Their research indicates that this was the first instance of calculating a CDR value directly from a fundus picture, without the need for segmentation.

S. C. Shetty and P. Gutte (2018) In order to distinguish between the optic cup and disc, the authors' study used K-means Clustering to group various visual components according to color. Ultimately, the boundary method's fractal dimension value may be used to detect glaucoma. With this method, the authors were able to distinguish between healthy eyes and those with glaucoma in 92% of instances. This approach may potentially be useful for detecting diabetic retinopathy.

## EXISTING METHODS

**Popular methods**

**Convolutional neural network**

The optimum deep learning model for glaucoma classification from retinal pictures is a CNN, which is very skilled at extracting and evaluating visual characteristics. CNNs automatically learn complex patterns indicative of glaucomatous alterations, beginning with low-level elements like as textures and edges and progressing to higher-level representations that are critical for diagnosis, thanks to their hierarchical design. They are more generalizable and resilient because they can locate important elements in pictures and use data augmentation methods. CNNs

trained on massive labeled datasets use backpropagation to accurately classify retinal pictures as either glaucoma or non-glaucoma, allowing for informed clinical decision-making. Ophthalmologists are able to help patients get the treatment they need sooner because to CNNs' data-driven automation, which improves glaucoma diagnosis.

**Recurrent Neural Networks**

Kind of deep learning models called RNNs excel in sequential data analysis, which makes them useful for glaucoma classification using longitudinal retinal imaging and other similar tasks. Unlike feedforward networks, RNNs may remember previous inputs and understand how data in sequences is dependent on one another over time because of their connections that loop back on themselves. To classify glaucoma, RNNs may examine retinal pictures captured over time at different appointments, revealing the gradual alterations that characterize glaucomatous disease. They are quite good at forecasting the course of diseases, identifying stable and advancing instances, and picking up on minor patterns of progression. To increase RNNs' performance in glaucoma classification, techniques such as Gated Recurrent Unit (GRU) architectures and LSTM algorithms deal with the vanishing gradient issue and enhance learning of long-term dependencies. Automated and reliable glaucoma diagnosis is made possible by RNNs because of its capacity to acquire contextual cues from sequential data and handle input sequences of varying lengths. This helps physicians with early intervention and individualized treatment plans.

**Artificial intelligent**

Machine learning and deep learning are two branches of AI that may be used to classify retinal pictures as indicators of glaucoma. Machine learning algorithms like SVMs, Random Forests, and Gradient Boosting Machines may analyse retinal pictures for characteristics and use those features to determine whether the images are glaucomatous or not. To accurately identify glaucoma, DL models, especially CNNs, shine when it comes to autonomously learning hierarchical features from raw picture data. To help with early diagnosis and prompt action, AI-based methods use big annotated datasets to build models that do well with unseen data. When it comes to glaucoma screening and care, ophthalmologists may greatly benefit from the automation, efficiency, and scalability that AI algorithms provide. In order to use AI in glaucoma classification responsibly and effectively, we still need to solve three major issues: the interpretability of model choices, integration into clinical processes, and ethical concerns.

## DISCUSSION

Ophthalmologists are facing a serious challenge in detecting glaucoma, a disorder that may cause irreparable visual loss if left untreated. However, there is hope that ML and DL approaches together have showed potential in this area. Following an overview of the anatomy of the eye and the pathophysiology of glaucoma, this study delves into the most recent developments in ML/DL-based methods for glaucoma identification. Important characteristics retrieved from retinal pictures are covered, along with several techniques such as Support Vector Machines, Random Forests, Convolutional Neural Networks, and Recurrent Neural Networks. Collaborative efforts are urged for standardized methods, bigger datasets, and regulatory compliance in light of highlighted challenges such as data scarcity, class imbalance, and model interpretability. For future breakthroughs in AI-driven glaucoma diagnosis and treatment, it is crucial to prioritize interpretability, ethical issues, and multidisciplinary cooperation.

## CONCLUSION

Finally, ophthalmic care stands to benefit greatly from the development of ML and DL approaches for glaucoma identification. The present state of ML/DL-based techniques has been investigated in this survey, and their ability to aid in the early detection and treatment of glaucoma has been highlighted. To guarantee the safe and successful integration of these technologies into clinical practice, however, a number of obstacles, including data scarcity, model interpretability, and ethical issues, must be overcome. To solve these problems and create scalable, interpretable, and morally acceptable AI-driven solutions for glaucoma diagnosis and beyond, it is vital for researchers, physicians,

industry stakeholders, and regulatory agencies to work together. To fully use AI for better patient outcomes and lessen the worldwide impact of glaucoma-related blindness, we must emphasize openness, data privacy, and regulatory compliance as we move towards this future.

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**Table 1: Comparison of Machine Learning Methods for Glaucoma Detection**

| Reference | Method | Advantages | Disadvantages | Accuracy |
|---|---|---|---|---|
| **1** | Mask Region-Based convolutional neural network (Mask-RCNN) | Mask-RCNN) shows a distinct pattern for eyes with glaucoma and those without, which might be used to identify glaucoma using machine learning. | This component enhances the location of the BB and assigns ROIs to many more categories than the RPN module. | **93%** |

| | | | | |
|---|---|---|---|---|
| 3 | convolutional neural network | A novel glaucoma classification was developed by merging many characteristics retrieved by various convolutional neural networks. | A clear benefit of classifier was that it uses more information for general picture categorization, even if the pathophysiology underlying it was hard to describe. | |
| 5 | ResNet-50 and GoogLeNet | Two deep learning algorithms, ResNet-50 and GoogLeNet, were used to classify fundus pictures as either early or advanced glaucoma. | Among the three methods for early glaucoma identification, optic disk examination was the most popular and commonly employed by experts due to its convenience. The fact that it was expensive and time-consuming was still a drawback. | ResNet-50 – 0.90% Googlenet – 0.91% |
| 11 | CNN | may include using same author methods on a broader and more varied collection of pictures | A large number of studies were carried out using glaucoma datasets that were made publically accessible. | 93% |

**Table 2: Comparative Analysis of Machine Learning Algorithms for Glaucoma Detection**

| Algorithm | Merits | Demerits |
|---|---|---|
| **CNN [1]** | It is no longer necessary to manually extract features from raw retinal pictures; CNNs can learn discriminative features automatically. When it comes to detecting tiny changes caused by glaucomatous disease, this expertise is invaluable. | To train well and generalize to unseen samples, CNNs usually need a lot of labelled data. It might be difficult to get annotated datasets with a wide range of patient demographics and disease severity levels for use in glaucoma classification. |
| **RNN [19]** | Longitudinal retinal imaging or the dynamic changes in glaucomatous characteristics over time are examples of time-series data that may be well-analyzed using Recurrent Neural Networks (RNNs) due to their exceptional ability to capture temporal relationships and sequences. | Because of the vanishing or exploding gradient issue, which occurs when gradients become either too tiny (vanishing) or too high (exploding) during backpropagation, training deep RNNs may be hard. This has the potential to impact model performance and make it harder to understand long-term dependencies. |
| **Artificial intelligent [10]** | When trained on enough and representative data, artificial intelligence (AI) techniques like ML and DL models may achieve excellent accuracy in glaucoma classification tasks. They may help with early diagnosis and management by identifying subtle patterns and characteristics of glaucomatous alterations in retinal pictures. | The amount and quality of training data have a significant impact on AI models. In real-world clinical settings with different patient populations, inferior performance and generalization concerns might be caused by biased or limited datasets. |

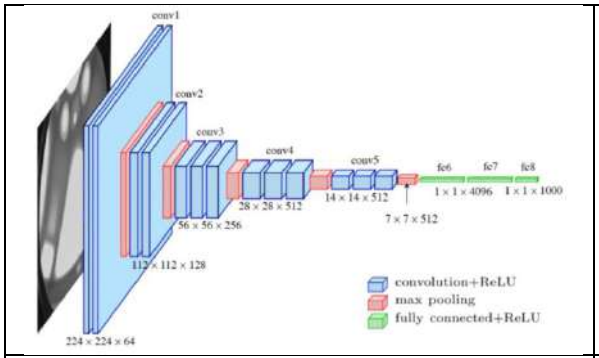


**Figure 1: CNN architecture [Source: https://vitalflux.com/different-types-of-cnn-architectures-explained-examples/]**

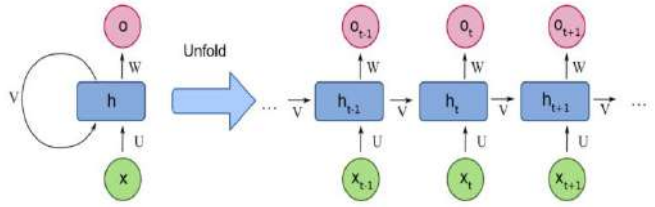


**Figure 2: [Source https://www.analyticsvidhya.com/blog/2022/03/a-brief-overview-of-recurrent-neural-networks-rnn/]**

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**RESEARCH ARTICLE**

# Bridging Heritage and Innovation in Sustainable Agriculture of India

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**ABSTRACT**

Sustainability is a key focus in the transition from traditional to contemporary technologies in Indian agricultural approaches, and a look into the past is necessary to set the stage for this change. The dynamic geography of agricultural practices investigated by the study illustrates an agricultural renaissance which points out how technology has been seamlessly incorporated alongside traditional techniques. This article also objectively assesses the ecological, economic and production effects of smart farming measures which include integrated pest management and technological advancements. It further explores what these changes could mean for country people at large, giving understanding on how they impact ways of life as well as commercial opportunities.

**Key words**: Sustainability, Traditional, Technology, Investigation, Economy.

## INTRODUCTION

This article looks at India's journey in agriculture from its ancient practices to the inclusion of contemporary technologies. It evaluates the revolutionizing process that traditional farming techniques have experienced; hence the shift that has occurred in Indian Agriculture. The article stresses the dynamic interaction between traditional wisdom and modern farming advancements, as revealed by Smith, 2020 & Jones, 2019 [1,2]. It examines how fusing indigenous knowledge with new technology has contributed to holistic sustainable agriculture [3]. By doing so, not only does it come up with a response to current challenges, but it goes beyond productivity enhancement by making agricultural systems more sustainable and resilient in terms of world markets [4].

Thus, this paper tracks various transformations and novelties which lead one into understanding Indian Agriculture better. It stresses the importance of combining tradition and technology for future agricultural development in India, thereby making it progressive and sustainable.

**Historical Context and Traditional Indian Farming Practices**

A profound understanding of traditional farming practices that have influenced the nation's agricultural backdrop for ages is possible by delving into the historical roots of Indian agriculture. To sustain high production and ecological balance, such ancient methods as crop rotation, intercropping as well as water conservation were practiced accord [5]. These strategies demonstrate the adaptation measures applied by Indian farmers towards different climatic and environmental conditions.

Crop rotation calls for planting various crops in different fields to enhance nutrient availability and conserve soil fertility. Conversely, intercropping allows farmers to cultivate several crops in one field consequently optimizing land use and reducing pests/disease pressure naturally. India's innovative techniques of water management can be seen through traditions such as rainwater harvesting and canal irrigation systems. These conventional agriculture practices formed the basis for contemporary agricultural technologies in India, with a significant impact on current strategies particularly those related to sustainable agriculture [6]. On recent investigations it was found that mixing old wisdom with new innovations is contributing towards resilience enhancement and sustenance of farming systems [7,8,9].

**Integration of Modern Technologies with Traditional Practices:**

Having the context changed from ancient to modern farming methods, we find the combination of modern cutting-edge innovation and the traditional principles of cultivation. A neat angle is the work of some scientist [10,11], who have been pioneers in precision agriculture. They write about drones that fly over fields, surveying the whole farming operation with just the National Aeronautics and Space Administration (NASA) image of the same area and the farmer's position, considering the environment, the return on investment, and the increase of agriculture and the quality of the crop.

Precision agriculture introduces us to a few remarkable developments that modify the old ways of farming. As a result of instruments like drones flying, satellite imagery, and sensor systems [12], among others, we have seen many great chances for precision agriculture. They can rationalize resource use and, therefore, don't harm the environment as much [13,14]. The recent years have witnessed the use of big data analytics and artificial intelligence (AI) algorithms in agriculture as well, that also entails real-time decision-making and predictive modeling [15,16]. Precision agriculture is not all about increasing the quantity and quality of the crops only. It also focuses on sustainability issues through smart pesticide and fertilizer management via an integrated system [17,18]. On top of that, it contributes to water-saving techniques by such actions as regulated irrigation and soil moisture tracking [19,20]. These breakthroughs make the point about how high-tech and traditional farming methods form a symbiotic relationship. This is an approach that will foster agricultural productivity and environmental stewardship at the same time.

**Various Landscapes of India and Farming:**

India's diverse topography has a major impact on the agricultural practices, such as the dry sands of Rajasthan and the fertile plains of Punjab. This means that India must deal with a situation where each region presents a different kind of problem that must be solved with various methods from choosing the right crops to adequate crop and soil management to water resource optimization strategies. Recent studies confirm the need to adjust agricultural techniques to environmental conditions and climate variability. For the sake of an example, research by Kumar et.al.(2016) [21] mentions such adaptable crop systems that ensure not only the production of crops consumed in people's area but that are also beneficial to the environment and economy of a certain area. Gupta et al. (2018) [22] promotes checking soil quality by integrating nutrient management practices to land the development of soil health. They have realized the benefits of sustainable soil fertility and soil health management technologies too.

The environment must also be considered with reference to water, as 'Mishra et al. (2023) [23] examined novel irrigation methods and the introduction of advanced water-saving technologies that can address the need to save water and increase food production. Technologies that can be named as such are wireless drone-based monitoring

systems [24] and sensor networks [25] that provide real-time information direct to the farmer, which in turn results in policy making for optimal and efficient resource utilization in agriculture. Applying the experimental research knowledge into local agriculture is the key tool necessary for combining the different landforms of India and reaching its goal. By introducing innovative geographical strategies that are based on the latest research and with this applying cutting-edge research, farmers will potentially perform sustainable food production while they maintain the balance of nature.

**Smart Farming Approaches: Integrated Pest Management and Beyond**

Smart farming is already working on modernizing agriculture, boosting food processing and the environment together with it. IPM, as per Sharma and Verma (2019) [26], basically means reduced dependence on chemical pesticides and higher agriculture productivity is achieved using bio-control agents, cultural practices, and targeted pesticide application.

Not only IPM, but smart farmings newest technologies also have something new to talk about artificial intelligence, data analytics, and precision farming. The study of Raj et al. (2020) [27] leaves no doubt that data analytics is a powerful tool for farm management decision making that can harness big data for building predictive models for real-time monitoring. AI in agriculture is the disruption that is best explained in the work of Gupta et al. (2018) [28] who find that it had been used for crop monitoring and yield prediction with the most accurate performance level. Forward, precision farming technologies, as studied by Patel et al. (2019) [29], are finding ways to marry observed space-time data with the inputs of water, fertilizers, and pesticides and remediate the environment. Hence, the outcome is the optimization of resource use and the reduction of environmental footprint in the region.

Furthermore, some innovations like sensor networks, automation tools, and IoT devices keep on improving the efficiency and sustainability of smart farming [16,13] more specifically, they provide agriculture experts with the opportunity to monitor soil, weather parameters, and the growth of plants in real-time, thus taking preemptive steps. The smart farm development is one of the agricultural expansions facilitated by technology, which through the implementation of modern technology, improves the agricultural process, while at the same time, ensuring the provision of quality products and taking care of the environment.

**Changing Livelihoods of Farmers and Rural Communities**

The agricultural revolution in India has occasioned some major changes in society and the economy. That is way more than what occurs on the farms and synthesizes to affect the existence and livelihoods of farmers and the rural community at large in society. To better understand this transformation, its impacts on society and the economy should be considered.

The research by Gupta et al. (2017) [30] elaborated on various dimensions of changes that agricultural modernization entails with respect to changes in patterns of income, employment dynamics, and access to resources in a rural community. It showed how technological progress empowers but challenges old ways of farming. Patel and Kumar in 2018 [31] highlights the transformational impacts brought about by agricultural modernization within the economy of rural areas. It majors in changes within market dynamics, agricultural productivity, and socioeconomic inequality. According to this study, there are disparities in adoption of technologies and the implications this has on rural development. Singh et al. (2020) [32] tries to elucidate how government policies at times frame strategies for agricultural modernization and their implications for rural livelihoods. Mishra et al. (2019) [33] study the socioeconomic dimensions of sustainable agriculture aimed at inclusive growth and equity in developmental processes within rural areas. Sharma et al. (2021), Rao et al. (2018), and Kumar et al. (2020) The multifaceted nature of agricultural transformation, encompassing the gradual adoption of technology, diversification of income sources, and building of resilience within rural communities, contributes significantly to the development and sustainability of agriculture [34,35,36]. These studies, taken together, underline the deep-rooted socio-economic changes into which India's agricultural modernization were embedded:

- Changing pattern of incomes and employment relations.
- Alterations in agricultural productivity and market dynamics.
- Disparities in the diffusion of technological changes and socio-economic inequality.
- Government policies can play in shaping modernization strategies.
- How progress toward sustainable and more inclusive agriculture is needed.

In interpreting this, we could get a full insight into the challenges and opportunities from the current agricultural transformation taking place in India.

**Agricultural Revolution and Increased Gross Domestic Productivity:**
The final section of the discussion deals with the far-reaching effects of India's Green Revolution. The macroeconomic picture, examining the relationship between rises in GDP and agricultural progress, is presented herein. The review offers how the blend of the manual with technological means has not only resulted in changes in agriculture but also substantially impinged on the general economic development in the country. Agarwal and Joshi (2016) [37] explain how transformations in agriculture, through innovative means can stimulate economic growth by improving productivity, integrating markets, and enhancing livelihoods in rural areas. Their work clearly showcases the anchoring role of technology in making farming more efficient and profitable.

Similarly, Singh et al. (2019) [38] provide a comprehensive review regarding the socioeconomic effects of agricultural modernization on income generation, employment dynamics, and poverty alleviation in the rural setting, citing that the advancements in farming practices are some of the means toward attaining sustainable economic growth and development in rural areas. The complex effects of agricultural modernization on national economic progress and rural well-being are highlighted by recent studies, which probe different facets of agrarian transformation such as technology adoption, market reforms, and policy matters [39,40,41]. Research [42,43,44] has highlighted how the interplay of government policies, trends in agricultural productivity, and resilience building shape the agricultural landscape of India. These intellectual inquiries underline the deep and far-flung effects of India's agricultural revolution on economic growth, rural development, and societal well-being.

## CONCLUSION

The agricultural revolution in India is a fine example of a promising journey from traditional practices to amalgamation with the finest of modern technologies, thus inspiring sustainability and economic growth. This has been an evolution that reflects the consensus between ancient farming wisdom and modern innovation. Crop rotation, intercropping, and conservation of water have been really deep within the agricultural culture of India, merging without a hitch with other innovations like precision agriculture and artificial intelligence. Prodigious farming today, using precision agriculture tools like drones, satellite imaging, and sensor-based systems, has fundamentally changed the face of farming with increased resource efficiency and reduced harm to the environment, using optimized crop management practices. This has increased productivity and supported sustainable agricultural practices in vast landscapes, from arid regions in Rajasthan to the fertile plains of Punjab.

Moreover, IPM has significantly minimized the dependence on chemical pesticides; such efforts have contributed impressively to an enhanced ecological balance and higher quality of crops. Indeed, such holistic approaches would not only ensure that the natural ecosystem is protected but also guarantee food security and resilience in the rural community. These socio-economic implications of India's agricultural modernization emphasize some fundamentally transformative changes in the patterns of income, employment dynamics, and access to resources across rural India. In this respect, the government policy would, therefore, turn out to be the mainspring of change in making this transformation more inclusive and market-oriented in terms of growth. Leaning on the wisdom of ancestral knowledge, wedded with strides in technology, the agricultural revolution in India will remain an example of paradigm-changing power. Therefore, building on a rich agricultural heritage, India has laid the foundation for

sustainable agricultural practices aimed at improving livelihoods, protecting the environment, and working toward economic prosperity.

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**RESEARCH ARTICLE**

# The Cardio Protective Potential of *Ficus arnottiana* Leaf Extract against Acute Myocardial Infarction in Rat Models

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**ABSTRACT**

This study investigates the potential cardioprotective effects and underlying mechanisms of *Ficus arnottiana* leaf extract (FAE) against acute myocardial infarction (MI) induced by isoprenaline in Wistar rats. Animals were categorized into control, MI, and MI with varying doses of FAE treatment groups. Evaluation encompassed myocardial enzymes, cytokines, and oxidative stress markers in both blood and isolated cardiac tissues. Compared to control and treatment cohorts, MI animals exhibited significant elevations in cardiac enzymes, pro-inflammatory cytokines, and oxidative stress markers, while FAE treatment attenuated these alterations in a dose-dependent manner. Moreover, FAE mitigated inflammation and fibrosis related changes, including expression levels of TNF-$\alpha$, TGF-$\beta$ and IL-6 in MI cardiomyocytes. These findings suggest FAE as a promising therapeutic agent against MI, potentially mediated through modulation of cardiac enzymes, cytokines and oxidative stress in cardiac tissue.

**Keywords:** *Ficus arnottiana* leaf, myocardial infarction, cytokines, anti-oxidants, oxidative stress, apoptosis.

## INTRODUCTION

*Ficus arnottiana*, a member of the moraceae family, is a botanical specimen characterized by its smooth, hairless (glabrous) appearance, presenting as either a tree or a shrub. Referred to colloquially as Paras Pipal, this species is widespread across the Indian subcontinent, flourishing particularly in regions characterized by rocky terrain and elevations reaching up to 1,350 meters above sea level. Its presence is notably prominent in rocky hillsides, where it

establishes itself as an integral component of the local ecosystem [1]. *Ficus arnottiana* leaves, known as Paras Pipal, have diverse medicinal applications such as fertility control, anti-inflammatory, digestive aid, skin conditions, antioxidant properties, respiratory health and menstrual disorders [2]. The leaves of the plant contain rutin, friedelin, taraxosterol, lupeol, β-amyrin along with psoralen, bergapten, β-sisterol and quercetin-3-galactoside [3].In a previous study, we found that *Ficus arnottiana* leaf extract showed cardioprotective effects against streptozotocin induced diabetic cardiomyopathy in rats[4], suggesting it may be beneficial for treating cardiovascular diseases. Cardiovascular disease is highly prevalent in the elderly. Acute myocardial infarction (MI), a kind of coronary heart disease, can be induced by isoprenaline(45mg/kg) in animals [5]. In the present study, we induced MI by giving a single dose of isoprenaline(45mg/kg)in rats and studied both the protective effects of *Ficus arnottiana* leaf extract (FAE) and the possible mechanisms involved.

## MATERIALS AND METHODS

### Plant Material

*F. arnottiana leaves* were identified taxonomically and authenticated by Dr. S.N.Dwivedi, a botanist from the Department of botany, Janta PG college, APS university, Rewa(M.P).

**Drugs and Chemicals**

Isoprenaline was acquired from Ranbaxy Research Laboratories in Gurgaon, India. Analytical grade chemicals, including a variety of organic solvents, were sourced from E. Merck India Ltd and Ranbaxy Laboratories in India for the extraction and phytochemical analysis of the constituents.

**Preparation of Plant Extracts**

The leaves were harvested from the plant and air-dried in the shade at room temperature. Subsequently, they were ground into a fine powder. A total of 450 grams of the powdered material was subjected to extraction using solvents of ascending polarity, starting with methanol. Each solvent was allowed to extract for 24 hours using a hot extraction method employing a Soxhlet apparatus, maintained at a temperature of 60 degrees Celsius. Following extraction, the solvents were concentrated under reduced pressure using a rotary evaporator until a constant weight was achieved. The resulting extracts were collected and stored in a desiccator to maintain their integrity until further analysis.

**Preliminary Phytochemical Study**

A portion of residue from methanolic extract was subjected to phytochemical analysis in order toidentify the presence of sterols, alkaloids, carbohydrates, tannins, phenols etc in the leaves extracts[6, 7].

**Determination of cardiac injury biomarkers, oxidative stress, cytokines and apoptosis markers level in acute MI rats**

**Animals**

A total of 30 male Wistar rats were used in this study and they were obtained from Central Animal House of our institution. Male Wistar ratsweighing ≈150–200 g, were housed under standard 12hr light/12hr dark conditions with free access to water and food. Rats were randomly divided into sixgroups. All procedures were performedin compliance with the Standard care was provided to the animals as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA) guidelines. All animals were allowed to acclimatize for one week before the experiment[8].

**Study Procedure**

**Acute myocardial infraction induction procedure**

The acute myocardial infraction model that we haveused in this study was isoprenaline induced myocardial infraction model[5]. Acute myocardial infraction was induced in male Wistar rats by giving isoprenaline injections

intraperitoneally. A single dose of isoprenaline (45 mg/kg) on the first day, followed with oral dosing of different doses of *F.Arnotianna* extract (100 mg/kg, 250 mg/kg, 500 mg/kg) for 7 consecutive days[9].

**Treatment Schedule**

Wistar rats were divided into five groups, each group had six rats. The study consist of total seven days in which a single dose of isoprenaline i.p (45 mg/kg) on the first day, then oral dosing of different doses of *F. arnotianna* extract (100 mg/kg, 250 mg/kg, 500 mg/kg) for 7 consecutive days. Treatment for all the groups was started on the 1st day cotreatment with isoprenaline . The treatment schedule of various groups were as follows: Normal control (NC) (normal saline, per oral), Negative control group, three test groups were treated with *F. arnotianna* extract of (100 mg/kg, 250 mg/kg, 500 mg/kg per oral) . Oral feeding of drugs was done using steel oral gavage tube (16 G, 7.6 cm)[9].

(1) Group 1, the control group (CON), the rats were orally feeded with normal saline (10 ml/kg) for 7 consecutive days.

(2) Group2, Negative control group (MI), the rats were intraperitonially injected with isoprenaline (45 mg/kg) on the first day and thenorally feeded with normal saline (10 ml/kg) for 7 consecutive days.

(3) Group3, the treatment group, the rats were intraperitonially injected with isoprenaline (45 mg/kg) on the first day and then orally feeded with *F. arnotianna* extract (100 mg/kg) for 7 consecutive days.

(4) Groups 4, the treatment group, the rats were intraperitonially injected with isoprenaline (45 mg/kg) on the first day and then orally feeded with *F. arnotianna* extract (250 mg/kg) for 7 consecutive days.

(5) Groups 5, the treatment group, the rats were intraperitonially injected with isoprenaline (45 mg/kg) on the first day and then orally feeded with *F. arnotianna* extract (500 mg/kg) for 7 consecutive days.

**Estimation of cardiac injury biomarkers**

CK-MB, LDH, and SGOT were estimated enzymatically in serum by commercially available kits (Erba Mannheim, Beacon Diagnostic and Accurex Biomedical Pvt.ltd, Mumbai, India) in accordance with the manufacturer's instructions.

**Analysis of oxidative stress parameter**

For oxidative stress parameters, cardiac tissue was homogenizedin an icecold medium of phosphate buffer saline (PBS) pH 7.4 with 3 mM EDTA using a homogenizer. Centrifugation at 7000 rpm for10 min at 4°, cell debris was removed using a refrigerated centrifuge to clear supernatants. Following that, protein estimation was performed to estimate protein concentration in supernatant as described by Lowry *et al*[10]. Prepared supernatants were analysed to determine malon dialdehyde (MDA)[11], nitrite[12], SOD[13], and CAT activity[14].All results were calculated as per mg of total protein content.

**QRT-PCR Analysis of Cardiac Tissue**

Total RNA extraction from cardiac tissue was performed using TRIzol reagent, followed by assessment of RNA purity and quality. Reverse transcription into complementary DNA (cDNA) was accomplished using NeoScript 1st strand cDNA Synthesis Kit. Subsequently, qPCR analysis was conducted using SYBR Green Eye-Ab Universal qPCR Master Mix in the Agilent AriaMx RT-PCR system. Thermal cycling conditions were optimized according to the manufacturer's recommendations. The 2-ΔΔCt method was employed for relative gene expression analysis, with 18S serving as the housekeeping gene. Primer sequences utilized in this study are provided. This standardized methodology ensures accurate and reproducible assessment of gene expression changes in cardiac tissue[15]. The primer sequences used in thisstudy are listed in Table 1.

**Histopathological Examination of Cardiac Tissues**

Cardiac specimens from all experimental groups were excised andpreserved in 10% neutral buffered formalin for fixation before processing[16].After that, tissue processing was done by washing the tissues with PBS and then dehydrating them in varying alcohol(ethanol) concentrations ranging from 70% to 100%. After that, tissues were cleared with xylene and embedded in paraffin to make tissue blocks. These tissue blocks were sectioned at a

thickness of 5 μm for histopathological study[17].The rehydrated tissue sections were stained with haematoxylin and eosin (H&E) for histopathological evaluation .

**Statistical Analysis**

One-way ANOVA followed by post hoc analysis with Tukey test was conducted to evaluate statistically significant differences in the study variables between treatment groups. SPSS version 21.0 was used to conduct statistical analysis in this study.

## RESULT

**Effect of *F. arnotianna* Extract on cardiac injury biomarkers**

CK-MB, LDH and SGOT are important clinical markers of cardiac injury (REF). Serum levels of CK-MB, LDH and SGOT were significantly elevated in the negative control group (MI) as compared with the control (CON). Treatment with the *F. arnotianna* Extract (100 mg/kg, 250mg/kg and 500 mg/kg ) group significantly reduced their levels as compared with the negative control group (MI).

**Effect of *F.Arnotianna* Extract on oxidative stress parameters**

MDA is a biomarker of lipid peroxidation [18]. The levels of MDA were significantly elevated in the negative control group as compared with the control. Treatment with the *F. arnotianna* Extract (100 mg/kg, 250mg/kg and 500 mg/kg) group significantly reduced their levels as compared with the negative control group (MI).There was a significant increase in the level of Nitric Oxide in the cardiac tissue of negative control group (MI) compared with the control group. In contrast, treatment with *F. arnotianna* Extract (100 mg/kg, 250mg/kg and 500 mg/kg) at all the doses showed a significant decrease in the level of the NO. The important endogenous anti-oxidants like superoxide dismutase, catalase and glutathione shield the tissue against oxidative stress ad free reactive oxygen species. In this study there was significant decrease in the SOD, CAT and GSH levels in the negative control group (MI). When compared to control, Treatment with the *F. arnotianna* Extract (100 mg/kg, 250mg/kg and 500 mg/kg) group significantly improved their levels as compared with thenegative control group (MI).

**Effect of *F.Arnotianna*Extract on TNF-α, TGF-β and IL-6 in cardiac tissue**

Inflammation plays a vital role in MI. Hence, we determined the mRNA expression of TGF-β[19] (Figure 9), TNFα[20] (Figure 10), and IL6[21] (Figure 11) in kidney tissue. The administration of isoprenaline resulted in significant upregulation of TGF-β , TNF-α and IL-6 in rat heart. Treatment with *F. arnotianna* extract at both doses (250 and 500mg/kg) showed significant downregulation of cardiacTGF-β , TNF-α and IL-6 inflammasome. These results demonstrated that *F. arnotianna* extract protects the heart from inflammation caused by isoprenaline induced acute MI.

**Effect of *F.Arnotianna* Extract on heart histology**

To assess the damage at the cellular level, we performed histopathology of the heart by H&E staining. Negative control group (MI) animals showed loss of myocardial fibre, lesions, vacuolation, and cardiomyocyte disarrangement, while the *F.Arnotianna*Extract showed improvement in the myocardial fibers, lesions, and vacuolation. Fig 12.Representative photomicrographs of rat heart stained with hematoxylin and eosin; (magnification: 40×); CONTROL: Control animals showing normal cardiac myocardial fibers bounded with endomysium, no vacuolation, necrosis or inflammation, cardiomyocytes surrounded with numerous blood capillaries; Negative control group(MI): showing loss of myocardial fibers with severe lesions and cytoplasmic vacuolated cells, cardiomyocytes disarrangement with congested blood vessel.; low dose *F. arnotianna* Extract (250mg/kg) treated group: showing reorganization of cardiac myofibers , small vacoules of some cardiomyocytes; Highdose *F. arnotianna* Extract (500mg/kg) treated group: showing cardiac muscle fibers of normal shape and size.

## DISCUSSION

Injury stemming from myocardial infarction arises from the inhibition of aerobic glucose oxidation, heightened anaerobic glycolysis, and the subsequent accumulation of lactic acid dehydrogenase. Concurrently, diminished ATP production, disruption of ionic gradients, and compromised membrane stability culminate in the extrusion of enzymes typically housed within cardiomyocytes. Consequently, elevations in serum myocardial enzymes serve as markers of myocardial ischemia-induced impairment. In this investigation, myocardial infarction (MI) rats exhibited notably higher CK-MB activity compared to control counterparts. Administration of low-dose Ficus arnottiana leaf extract (FAE) demonstrated significant efficacy in mitigating this rise in CK-MB activity when juxtaposed with the MI group ($p< 0.001$). Furthermore, medium and high doses of FAE exhibited significant protective effects, resulting in a marked decrease in CK-MB activity relative to the MI group ($p < 0.001$). Similarly, the activities of lactate dehydrogenase (LDH) and serum glutamic oxaloacetic transaminase (SGOT) were significantly elevated in the MI group compared to normal controls ($p < 0.001$). FAE exhibited a dose-dependent protective response against the surge in LDH and SGOT activities in MI rats.

These findings suggest that the observed protective effects against myocardial infarction-induced damage may stem from FAE's capacity to reduce enzyme activity, enhance cardiomyocyte membrane stability, and mitigate enzyme extrusion. TNF-$\alpha$, TGF-$\beta$, and IL-6 are pivotal mediators of inflammatory responses, exerting diverse biological effects. Their primary function involves orchestrating inflammatory reactions, which can precipitate tissue and organ dysfunction by triggering cardiomyocyte death, apoptosis, left ventricular dilation, and thinning of the left ventricular wall. Elevated levels of TNF-$\alpha$, TGF-$\beta$, and IL-6 are commonly associated with myocardial infarction and oxidative stress.Our findings revealed heightened concentrations of TNF-$\alpha$, TGF-$\beta$, and IL-6 in MI rats compared to normal control rats ($p < 0.001$), indicative of their involvement in cardiac muscle remodeling. Notably, Ficus arnottiana leaf extract (FAE) effectively countered the elevation of TNF-$\alpha$, TGF-$\beta$, and IL-6 levels. These results imply that FAE exerts regulatory control over MI-induced inflammation by attenuating the release of pro-inflammatory cytokines. Malondialdehyde (MDA), a by product of lipid peroxidation, poses a threat to cell proliferation and protein expression by forming Schiff bases upon reaction with free amino groups of proteins and nucleic acids. This process leads to crosslinking of biological macromolecules, culminating in degradation of myocardial cell membrane integrity. Consequently, the physiological functions of the heart are hindered, predisposing to severe arrhythmias and cellular necrosis. Superoxide dismutase (SOD) and catalase play critical roles as antioxidant enzymes, scavenging superoxide anions to prevent cellular damage and maintain the delicate balance between oxidation and antioxidation. Our findings unveiled an elevation in MDA levels and a decline in SOD and catalase activity induced by myocardial infarction (MI), indicating heightened oxidative stress and an imbalance between oxygen free radical production and scavenging in MI-afflicted rats.

*Ficus arnottiana* leaf extract (FAE) demonstrated a remarkable capacity to mitigate MDA production while enhancing SOD and catalase activity. This suggests that FAE functions as an antioxidant, fortifying the system tasked with scavenging endogenous oxygen free radicals. By inhibiting oxidative stress, particularly lipid peroxidation, FAE safeguards cardiomyocytes from oxidative damage, thereby bolstering myocardial health. In conclusion, Ficus arnottiana leaf extract (FAE) exhibited robust protective effects against several detrimental aspects of myocardial infarction (MI) in rats. These protective effects encompassed the preservation of cytomembranes, prevention of myocardial enzyme leakage, maintenance of antioxidant defenses, and modulation of TNF-$\alpha$, TGF-$\beta$, and IL-6 levels. Notably, some of FAE's protective effects surpassed those of conventional standard drugs. Mechanistic insights indicated that FAE's protective action may involve the regulation of apoptosis-related genes such as Bcl-2 and Bax, as well as the critical apoptotic enzyme, caspase-3. These findings underscore the potential of FAE as an effective agent for the prevention and treatment of MI and other cardiovascular diseases. While this study provides valuable insights, limitations such as financial constraints hindered the exploration of additional parameters necessary for a comprehensive understanding of FAE's protective mechanisms in MI. Future research endeavors should aim to

address these limitations and delve deeper into elucidating the intricate mechanisms underlying FAE's cardioprotective effects, thereby enhancing our knowledge and therapeutic approaches in cardiovascular medicine.

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**Table. 1 List of Primer Sequence.**

| S.no. | Gene | Primer sequence 5′ to 3′ | Accession no. |
|---|---|---|---|
| 1. | TGF-β | Forward: CAACACCAGCTGCTACCTCA | NM_001393707.1 |
| | | Reverse: GAGCTCGTCCTCATTCTCGG | |
| 2. | TNF-α | Forward: ACGTCGTAGCAAACCACCAA | NM_012675.3 |
| | | Reverse: AAATGGCAAATCGGCTGACG | |
| 3. | IL-6 | Forward : AGAGACTTCCAGCCAGTTGC | NM_031004.2 |
| | | Reverse: ACAGTGCATCATCGCTGTTC | |
| 7. | 18S rRNA | Forward: GCAATTATTCCCCATGAACG | NR_04 6237.1 |
| | | Reverse: AGGGCCTCACTAAACCATCC | |

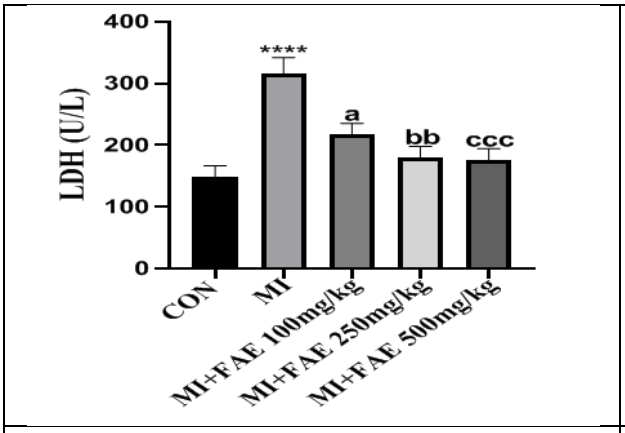


**Fig:1 LDH level where p****< 0.0001 compared to CON; $p^{a}$< 0.1 compared to MI; $p^{bb}$< 0.01,$p^{ccc}$< 0.001 compared to MI**

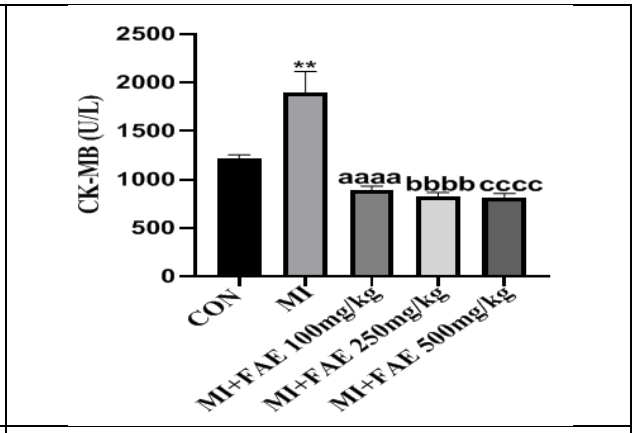


**Fig:2 CK-MB level where p**< 0.01 compared to CON;$p^{aaaa}$< 0.0001 compared to MI; $p^{bbbb}$< 0.0001,$p^{cccc}$< 0.0001 compared to MI**

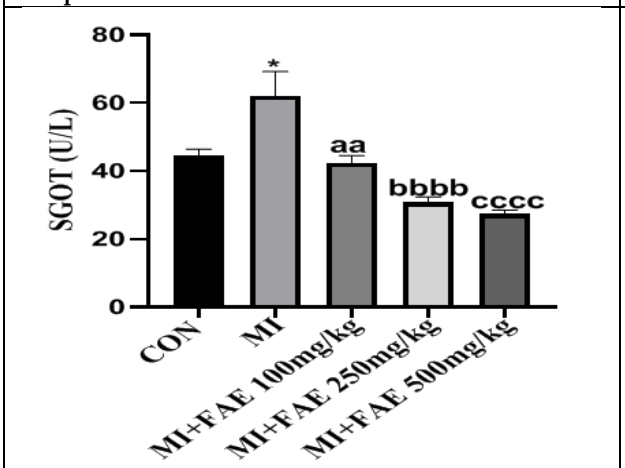


**Fig:3 SGOT level where p*< 0.1 compared to CON;$p^{aa}$< 0.01 compared to MI; $p^{bbbb}$< 0.0001,$p^{cccc}$< 0.0001 compared to MI**

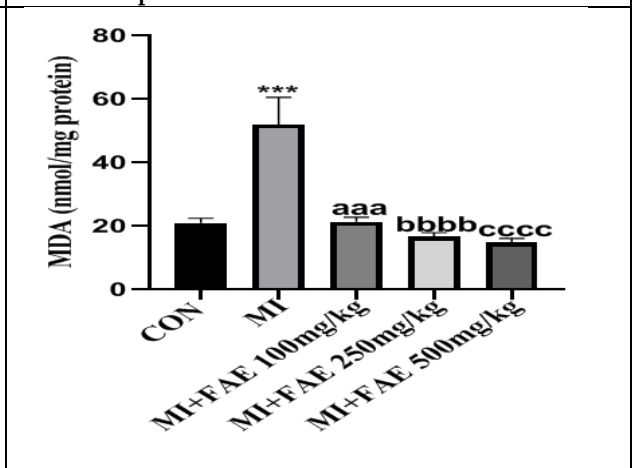


**Fig:4 MDA level where p***< 0.001 compared to CON;$p^{aaa}$< 0.001 compared to MI; $p^{bbbb}$< 0.0001,$p^{cccc}$< 0.0001 compared to MI**

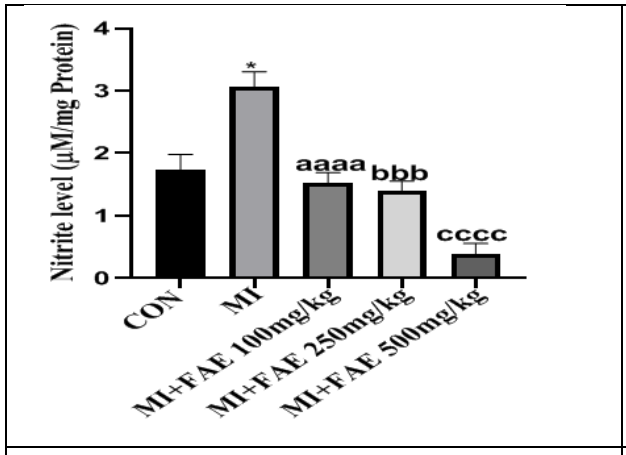


**Fig:5 Nitrite level where p*< 0.1 compared to CON;$p^{aaaa}$< 0.0001 compared to MI; $p^{bbb}$< 0.001,$p^{cccc}$< 0.0001 compared to MI**

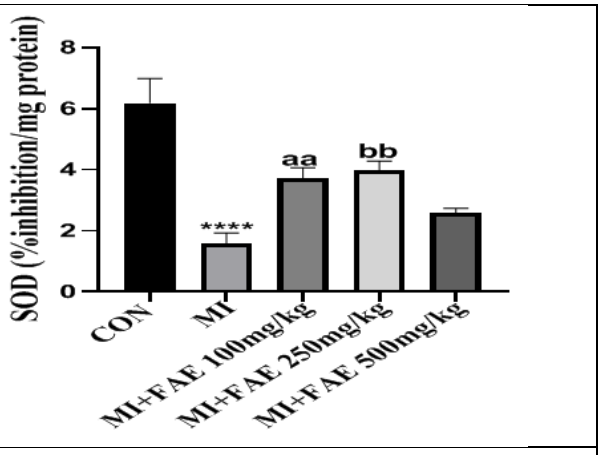


**Fig:6 SOD level where p****< 0.0001 compared to CON;$p^{aa}$< 0.01 compared to MI; $p^{bb}$< 0.01 compared to MI**

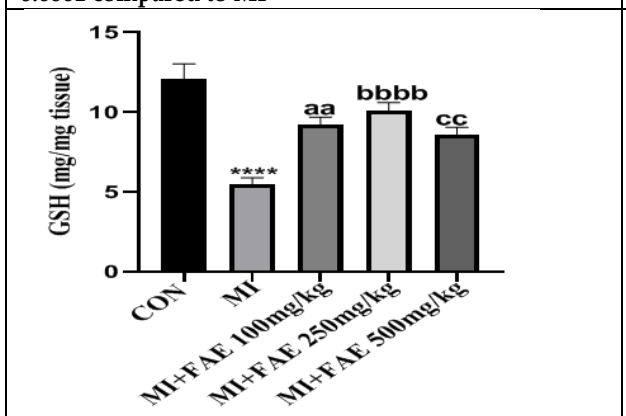


**Fig:7 GSH level where p****< 0.0001 compared to CON;$p^{aa}$< 0.01 compared to MI; $p^{bbbb}$< 0.0001,$p^{cc}$< 0.01 compared to MI**

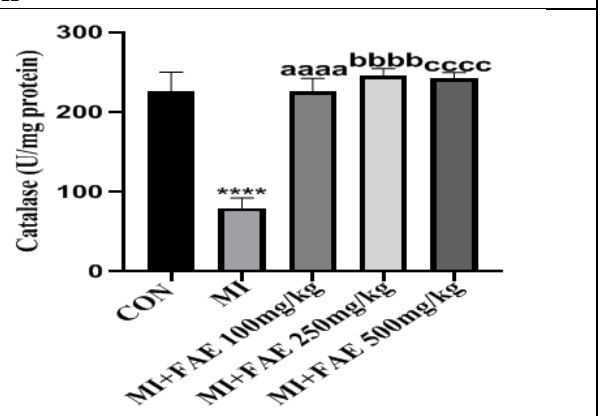


**Fig:8 Catalase level where p****< 0.0001 compared to CON;$p^{aaaa}$< 0.0001 compared to MI; $p^{bbbb}$< 0.0001,$p^{cccc}$< 0.0001 compared to MI**

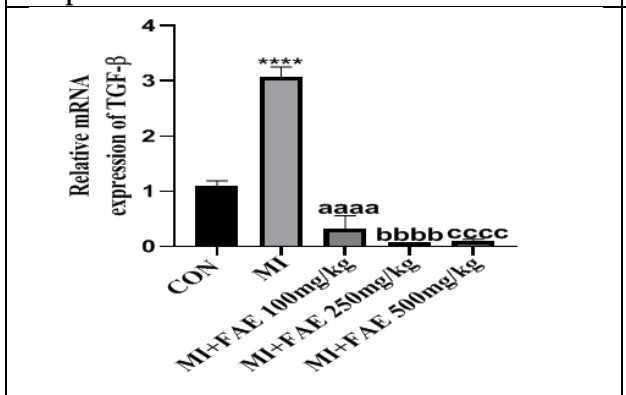


**Fig:9Relative mRNA expression level of TGF-β where p****< 0.0001 compared to CON;$p^{aaaa}$< 0.0001 compared to MI; $p^{bbbb}$< 0.0001,$p^{cccc}$< 0.0001 compared to MI**

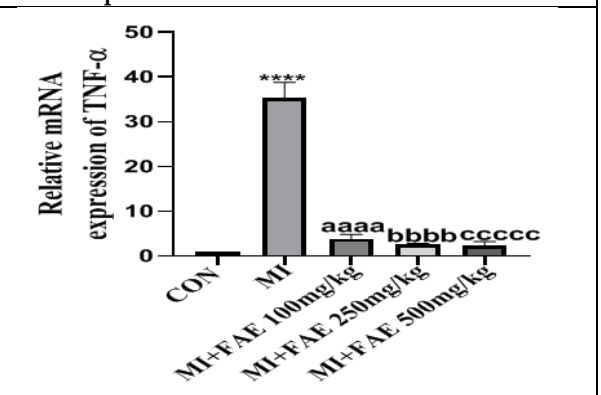


**Fig:10 Relative mRNA expression level of TNF-α where p****< 0.0001 compared to CON;$p^{aaaa}$< 0.0001 compared to MI; $p^{bbbb}$< 0.0001,$p^{cccc}$< 0.0001 compared to MI**

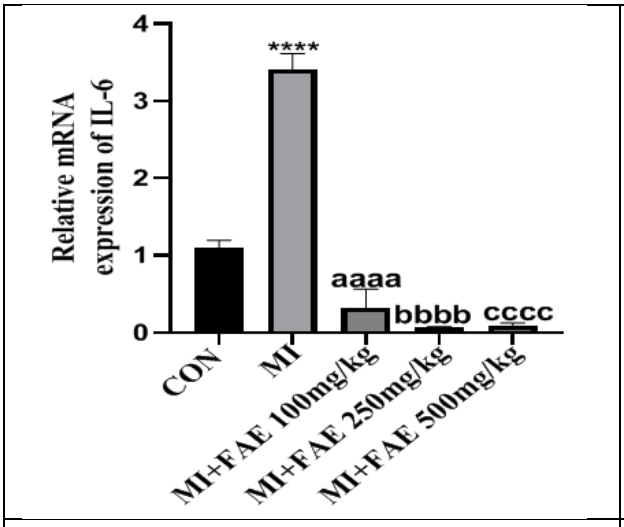


**Fig:11 Relative mRNA expression level of IL-6 where p**** < 0.0001 compared to CON; $p^{aaaa}$ < 0.0001 compared to MI; $p^{bbbb}$ < 0.0001, $p^{cccc}$ < 0.0001 compared to MI**

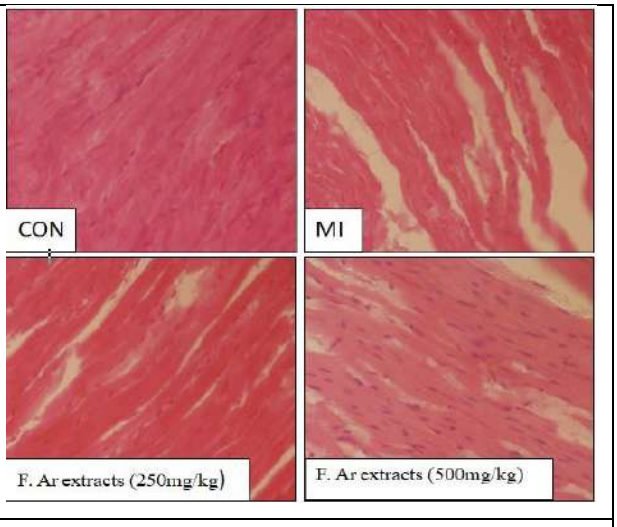


**Fig 12.Representative photomicrographs of rat heart stained with hematoxylin and eosin; (magnification: 40×);**

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**RESEARCH ARTICLE**

# Post-Partum Canine Mastitis in Golden Retriever Bitch

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## ABSTRACT

Canine mastitis is an unusual illness affecting the post-partum bitches 1- 10 days after whelping, which can cause severe illness starting with swelling/redness of the mammary gland, fever, inappetence to anorexia, lethargy, and vomition, when untreated, can lead to septicemia and death. However, the incidence and the risk factors of canine mastitis have not been well-studied. A 1.5-year-old Golden Retriever presented with a history of whelping 2 days before the expected date with clinical signs of inappetence, weakness, vomition, high temperature (104.3°F), slightly pale mucous membrane, and swelling of the mammary gland with reddish discharge on palpation. The discharge was collected using a sterile swab and subjected to isolation and identification of the causative agent using PCR and further subjected to a culture sensitivity test (CST) to determine the best treatment regimen.

**Keywords**: Canine mastitis, post-partum, CST, *Streptococcus*, PCR, hematobiochemical.

## INTRODUCTION

Postpartum illnesses in bitches, such as metritis and mastitis, can cause severe illness and may impair the ability to milk and care for the puppies [1,2]. Clinical mastitis in dogs can manifest in many different ways; the acute form usually appears during the postpartum phase. However, the illness might also manifest as a chronic, subclinical condition. There are a few suggested risk factors for canine mastitis, including systemic illness, trauma (typically from pups),and unhygienic surroundings [3,4].The illness usually strikes between the 6$^{th}$ and 10$^{th}$ day following whelping, with the postpartum phase being the most common time for it to occur [5,6]. It can also happen after early puppy weaning and during pseudo-pregnancy. The majority of the time, infections are ascending, while they can also spread hemorrhagically from other infected locations, like the uterus. Numerous bacteria, including *Streptococcus* species, *Escherichia coli*, and *Staphylococcus spp*., have been implicated as the disease's aetiological agents; yet, in certain cases of clinical mastitis, no microorganisms could be identified [7,8].

Mammary gland congestion is another condition that is assumed to be a risk factor for developing mastitis, while there is no proof of a link between the two conditions. Mammary gland congestion refers to the condition that is characterized by firm and engorged mammary glands without any changes to the milk, such as milk discoloration, a caseous appearance to the secretions, or other symptoms of mastitis. However, congestion of the mammary gland can also be a clinical symptom of mastitis[4,9]. Treatments for mastitis might be empirical, particularly in cases where prompt medical attention is required, or they can be based on the causative agent's culture and antibiotic susceptibility. *Staphylococcus spp*., *Staphylococcus intermedius*, *Staphylococcus haemolyticus*, β-hemolytic *Streptococcus*, *Klebsiella pneumoniae*, and *Escherichia coli* are among the bacteria that are frequently recovered from milk samples from bitches with mastitis [10].

**Case history**

A 1.5-year-old Golden Retriever was presented at the Division of Teaching Veterinary Clinical Complex with a history of whelping 2 days prior to the due date with clinical signs of in appetence, weakness, vomiting, high temperature (104.3°F), slightly pale mucous membrane, and swelling of the mammary gland with reddish discharge noticed. The dog was timely dewormed and vaccinated. The body weight of the bitch was 32 kg. Physical examination of mammary glands showed warm, oedematous, and painful conditions with palpable softs welling (Figure 1).

## MATERIALS AND METHODS

**Collection of Sample**

Collection of sample was done by squeezing purulent exudate from affected teats, which was collected in an Eppendorf tube and checked for pH using pH paper, and one more sample was collected directly from the teat with the help of a sterile swab and subjected to bacteriological examination and cultural sensitivity test (Figure 1).

**Bacteriological Examination**

The sample was processed for bacteriological examination by inoculating the milk sample in the nutrient broth and incubated at 37 °C for 24 hours. The inoculum was then used for streaking on the surface of brain heart infusion (BHI) agar plate using inoculating loop. Later the formed colonies were subjected for Gram's staining. The smear of suspension was prepared on a clean slide with a loopful of sample bacteria. Crystal violet stain was poured and kept for about 30 seconds and rinsed with water followed by pouring gram's iodine for 1 minute and decolouriser was used for about 10-20 seconds and rinsed with water. At last safranin stain was used for 1minute. Washed and air-dried slide was then observed under microscope.

**Molecular Characterization by Polymerase Chain Reaction (PCR)**

The isolate was further subjected for molecular diagnosis using PCR(initial denaturation, denaturation, annealing, 30 cycles, extension and final extension)against the *Strepagal* gene (F-5'CTGTGAGATGGACCTGCGTT and R – ACGCCCAATAAATCCGGACA 3') with the optimized PCR condition as follows, initial denaturation 94ºC for 2minutes, denaturation 94ºC for 15seconds, annealing 58ºC for 30seconds, 30 cycles, extension 72ºC for 45seconds and final extension 72ºC for 5minutes followed by gel electrophoresis.

**Culture Sensitivity Test**

Culture sensitivity test was done using disc diffusion assay against the following antibiotic disc: Oxacillin (OX, 1mcg), Gentamicin (GEN, 10mcg), Co-trimoxazole (COT, 25mcg), Ceftriaxone (CTR, 30mcg), Amoxicillin (AMC, 30mcg), Cefoperazone (CPZ, 50mcg), Doxycycline (DO, 30mcg), Penicillin G (P, 10units), Ampicillin (AMP, 10mcg) and Enrofloxacin (EX, 5mcg) (HiMedia, India).

**Hematobiochemical Test**

Hematobiochemical changes of the bitch before and 5 days after treatment were also studied for better understanding of the case. Blood was collected from the animal and subjected for complete blood count test (CBC test) that measured various parameters like hemoglobin(g/dL), total leucocyte count($10^3$/$mm^3$), mean corpuscular volume (fL), platelets count($10^3$/$mm^3$), neutrophils(%), lymphocytes(%), monocytes(%), eosinophils (%) etc using MYTHIC 18 VET Haematology Analyser, (Compact diagnostics India Pvt Ltd.). Biochemical parameters including total protein(g/dl), albumin(g/dl), globulin(g/dl), ALT(U/L), AST(U/L), BUN(mg/dl) and creatinine(mg/dl)were estimated by UV spectrophotometer using Erba diagnostic kits (Transasia Biomedical Ltd. Mumbai, India).

## RESULTS AND DISCUSSION

**Bacteriological Examination**

In this present study, the pH of mastitis milk was revealed to be alkaline (7.8), which was on par with previously conducted studies[1,11,12,13,2,14,15] where all quoted a high alkalinity of pH (7-9.5). In the case of mastitis, the pH level can increase due to infections such as *Streptococcus* or *Staphylococcus aureus*. This change in pH can make it more challenging to distinguish between contaminated milk and healthy mammary secretions. Additionally, severe mastitis is characterized by symptoms such as redness, hot milk, or discharge through one or more pores in the teat, which requires immediate attention and treatment from a veterinarian. So, without wasting any time, the sample was processed for bacteriological examination; the isolate revealed white pinpoint colonies on brain heart infusion (BHI) agar (Fig. 3), and on gram's staining, it showed a bunch of grapes appearance with purple color cocci, proving gram-positive. (Fig. 4).

**Polymerase Chain Reaction**

The isolate was further subjected to molecular diagnosis using PCR against the *Strepagal* gene (F-5'CTGTGAGATGGACCTGCGTT and R – ACGCCCAATAAATCCGGACA 3') with the optimized PCR condition as follows, initial denaturation 94ºC/ 2min, denaturation 94ºC/ 15sec, annealing 58ºC/30sec, 30 cycles, extension 72ºC/ 45sec and final extension 72ºC/ 5min followed by gel electrophoresis. The PCR result showed positive for the *Strepagal* gene at 352 bp (Figure 5), proving that the isolated bacteria is *Streptococcus spp.,* which was analysed and confirmed using gel electrophoresis. Our results were in concordance with a previously conducted study which reported *Streptococcus canis* was the organism isolated that was responsible for the mastitis [16].Some also mentioned that *E. coli, Staphylococcus sp., Streptococcus sp*., and other coliformsare the main infective agents responsible for thedevelopment of mastitis [17-19].

**Culture Sensitivity Test**

Culture sensitivity test using disc diffusion assay against the following antibiotic disc: Oxacillin (OX, 1mcg), Gentamicin (GEN, 10 mcg), Co-trimoxazole (COT, 25 mcg), Ceftriaxone (CTR, 30 mcg), Amoxicillin (AMC, 30 mcg),

Cefoperazone (CPZ, 50 mcg), Doxycycline (DO, 30 mcg), Penicillin G (P, 10 units), Ampicillin (AMP, 10 mcg) and Enrofloxacin (EX, 5 mcg) (HiMedia, India) revealed that Oxacillin and amoxicillin showed resistant and other remaining antibiotic disc showed susceptibility with zone of inhibition ranging from 26 mm – 35 mm.

**Haematological Analysis**

When it comes to the results of hematological analysis, it revealed mild anemia, a decrease in MCV, leukocytosis, neutrophilia, and thrombocytopaenia [20,21] where they reported leukocytosis and normocytic normochromic anemia while serum biochemistry revealed hyperproteinaemia, hyperalbuminemia, and hyperglobulinemia (Table 2 and figure 6) were on par with previous studies[22-25] where all reported increased ALT, AST, creatine, BUN, proteinogram and the values returned within the reference range on day 5 post-treatment.

**Treatment**

The results of culture sensitive test played a major role in choosing the choice of antibiotics in this case. Intravenous injection of Ceftriaxone (Intacef TazoPet$^{R}$ – 562.5mg) @ 25mg/kg body weight for 5 days with fluid therapy (Normal Saline @ 250ml and Ringer's Lactate @250ml)was given. Besides antibiotic therapy, supportive therapy was also given, which includes Inj. B- complex (Tribivet$^{R}$) @1ml, Inj. Flunixin meglumine (Megludyne$^{R}$) @ 1.1mg/kg body weight and Inj. Pantoprazole (Pantop$^{R}$) @1 mg/kg body weight intravenously. Manual emptying of the affected mammary glands was also carried out after fluid therapy, and advised to do the same at their home, too. A gradual reduction in the swelling of the mammary gland and improvement in the overall health of the animal started to be noticed on the day 2$^{nd}$ of treatment. Complete recovery of the animal's health noticed on day 5$^{th}$ of the treatment (Figure 4).

## CONCLUSION

Mastitis rarely occurs in lactating female dogs, but if left untreated, it can have serious effects on both the mother and her nursing puppies. This case study provides a comprehensive overview of canine mastitis, including clinical symptoms and treatment options, to help veterinarians approach the condition systematically rather than relying on general or symptomatic treatments.

**Conflict of Interest**

None of the authors have any conflict of interest to declare.

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**Table 1: Culture sensitivity test**

| S. No. | Antibiotic disc | Zone of Inhibition (mm) |
|---|---|---|
| 1 | Oxacillin (OX, 1 mcg) | 10mm |
| 2 | Amoxycillin (AMC, 30 mcg) | 10mm |
| 3 | Co-trimoxazole (COT, 25 mcg) | 26mm |
| 4 | Enrofloxacin (EX, 5 mcg) | 27mm |
| 5 | Ampicillin (AMP, 10 mcg) | 33mm |
| 6 | Doxycycline (DO, 30 mcg) | 29mm |
| 7 | Ceftriaxone (CTR, 30mcg) | 30mm |

| | | |
|---|---|---|
| 8 | Gentamicin (GEN, 10 mcg) | 26mm |
| 9 | Cefoperazone (CPZ, 50 mcg) | 30mm |
| 10 | Penicillin (P, 10 mcg) | 35mm |

**Table 2: The Hematobiochemical Parameters on Day 0 and Day 5**

| Parameters | Day 0 | Day 5 | Reference Range |
|---|---|---|---|
| Hb (g/dL) | 11.2 | 13 | 12-18 (g/dL) |
| TLC ($10^3$/mm$^3$) | 15.2 | 12.6 | 5.7-14.2 ($10^3$/mm$^3$) |
| MCV (fL) | 66 | 72 | 60-77 (fL) |
| Platelets count ($10^3$/mm$^3$) | 200 | 342 | 200-500 ($10^3$/mm$^3$) |
| Neutrophils (%) | 88 | 80 | 43-80 (%) |
| Lymphocytes (%) | 10 | 13 | 14-45 (%) |
| Monocytes (%) | 03 | 03 | 2-9 (%) |
| Basophils (%) | 01 | 0 | 0.1-1.1 (%) |
| Eosinophils (%) | 10 | 06 | 1-18 (%) |
| Total Protein (g/dl) | 8.6 | 5.6 | 5.4-7.5 (g/dl) |
| Albumin (g/dl) | 3.6 | 2.7 | 2.3-3.1 (g/dl) |
| Globulin (g/dl) | 4.5 | 03 | 2.40-4.40 (g/dl) |
| ALT (U/L) | 132 | 96 | 10-109 (U/L) |
| AST (U/L) | 21 | 15 | 9-49 (U/L) |
| BUN (mg/dl) | 20 | 16 | 5-21 (mg/dl) |
| Creatinine (mg/dl) | 1.1 | 01 | 0.5-1.7 (mg/dl) |

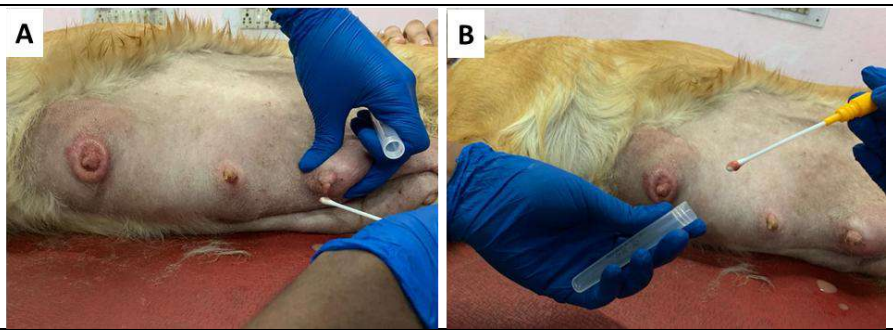


**Figure 1: Image A & B: showing the collection of mastitis milk samples for bacteriological examination.**

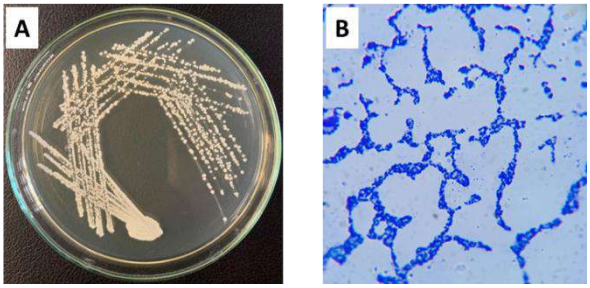


**Figure 2: Image A: Colony morphology showing white pinpoint, opaque, smooth, and circular colonies on brain heart infusion (BHI) agar. Image B: Gram staining showing characteristic bunch of grapes appearance with purple colour cocci, proving gram-positive.**

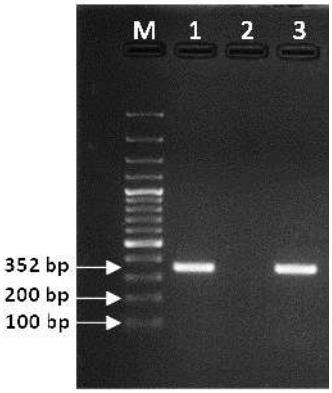


**Figure 3: PCR amplification results of strepagal gene of Streptococcus isolated from canine mastitis sample, analysed using 2% agarose gel electrophoresis stained with ethidium bromide. M- 100bp DNA marker; Lane 1- PCR product of strepagal gene (232 bp); Lane 2- negative sample; Lane 3- positive sample**

**Figure 4: Shows the culture sensitivity test performed on the isolated bacteria against 10 antibiotic discs.**

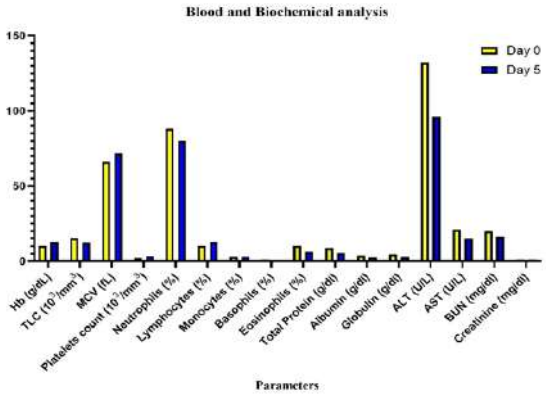


**Figure 5: The graph showing the changes in Haemato- Biochemical parameters between Day 0 and Day 5.**

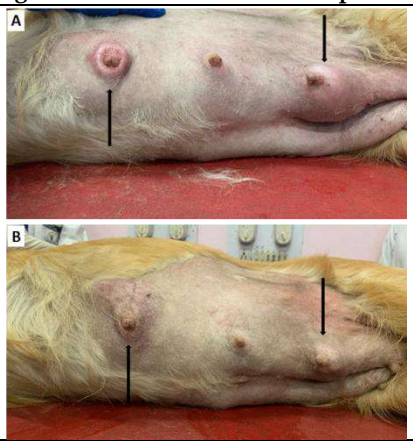


**Figure 6: Image A: Showing the inflamed and swollen mammary glands of the affected bitch before treatment (day 0). Image B: Showing the reduction in the inflammation and swelling of the affected mammary gland after treatment (day 5).**

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**RESEARCH ARTICLE**

# Investigating Environmental Education, Awareness, and Ethics among Teachers in Higher Primary Schools: A Case Study in Hassan District, Karnataka, India

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## ABSTRACT

Global warming and contamination of the environment are currently major global issues. Measuring environmental education, awareness, and environmental ethics among, higher primary school teachers at various schools in city of Hassan, Karnataka is the aim of the research project. As the questionnaires were created and distributed, the results of a sample of 42 Teachers -64% percent of whom were Male and 36% of whom were women-were examined in the current research study. The objective of the survey was to determine the Teachers level of environmental awareness. Three sections-attitude regarding environment, environmental awareness, and environmental education-each contained 138 prepared questions. The statistical analysis of the organized future study will be focused on environmental awareness. Simple random sampling methods are used to determine the sample size. Each of the characteristics of the environmental factors for every single item are shown using statistical analysis. When measuring inside consistency-a measure of how closely related a set of items is to one another-Cronbach's alpha indicated that the questionnaire had reached a level of satisfactory reliability. IBM SPS 22 is used to assess internal consistency. Upon analysing a sample of survey responds, it came out that around 85% of Teachers had provided positive feedback. The responses they gave focused on

minimizing environmental pollution and enhancing the state of the environment. In brief, a strong suit for Teachers is their awareness of environmental issues, which may be beneficial to our planet's health.

**Keywords:** Global warming, teachers, health, environmental, education.

## INTRODUCTION

The worlds environment has changed dramatically over the past few decades with certain modifications being directly attributed to human activity based on abbas 2013 the environment has been impacted at an alarming rate due to reducing air water and soil quality rising sea pollution wildlife extinction biodiversity loss and an increase in the intensity and frequency of catastrophic natural disasters which lead to property damage and fatalities. Worldwide climate change and the rate that the planets resources are being depleted exceed each other bozoglu et al 2016 further education ultimately damages environmental issues since it generates the leaders of tomorrow who will have authority to make choices over a wide range of issues in society graduates from these institutions are therefore expected to dealwith sustainability challenges in both their private and professional lives students have to develop a suitable diversity of environmental awareness cognition and concepts while they are in school in order to develop critical thinking skills (Corncoran and wals) 2004. The biological, physical, social, and cultural settings in which all living things coexist and interact with one another are referred to as the "environment." The reckless use of the planet'sresources and the unchecked destruction of its ecosystem, which have disastrous direct and indirect effects, are among the most important aspects of human existence. Numerous topics are covered under the broad heading of environmental health, including generation, pollution of the air and water, and climate change. While advancements in science and technology mayimprove our lives, they frequently have a negative effect on the environment. Human behavior is the major source of ecological issues, and only civilization can provide anexplanation. the environments delicate balance has been upset by human attempts to regulate it leading to harsh environmental issues that threaten human survival (GOK and kilie 2021). The global general awareness of the adverse effects that ecologically conscious production and environmental teaching and research destruction have on as well as the connections between the two has grown considerably over the last thirty years nowadays national educational policies curriculum design documents and conservation work all incorporate the concept of environmental education. Understanding the detrimental effects that human activity has on the environment and making steps to lessen or prevent these effects are components of environmental awareness.

A few hints that the method to evaluate the scope of the work we do has to be started. In this regard, the North American Academy for Environmental Education is currently attempting on creating "Standards" for the subject; yet, opposition of this concept understand it will more likely help to reduce speech than help promote it. Deterioration of the environment caused by undesirable mining practices, loss of biodiversity, storms draughts, and lack of sanitation are a few instances of man-made issues with the environment that jeopardize capacity of the natural landscape to sustain human life. Whereas some of these attempts are improving the country's food supply, they requires to be reined in to prevent harming the ecosystem and jeopardizing sustainability in the longer term. With a focus on introducing environmental education into the educational program, research shows that schools continue to experience human resources problems with balance in boththe teaching and support departments besides a lack of funds. While materials are needed to reduce the teacher ratio during guidance so that children can get sufficient time from their teachers, this can be barriers to the teaching of guidelines, their implementation, and environmental education. This element may assist students realize protection and practical methods to prevent pollution ( Mashaba 2022 ). Even the fact that every person deserves access to an ideal life, here are several components to today's society which make it challenging to achieve a satisfying one. Trouble may result with rising pollution of the environment in major levels. Individuals just struggle and are dissatisfied given that we lack concern about community as a whole, neither do we have an ethics or sense of responsibility for maintaining an optimal

environment. sincerely are interested in aiming for an improved standard of life that provides independence against sickness, hunger, and stress, then have to work together in reducing the planet's rising pollution (Chavada and Dinesh 2020). Along the period of the 21st century, the study of environmental ethics grew in size. A wide variety of opposing viewpoints can be supported by discussing the main worries in environmental ethics, whether to deal with such problems, as well as how environmental ethical thought applies with relevant practical problems topics in environmental ethics nowadays. Below that, we have present just a few of the essential, though completely opposite, issues of objections in the topic of environmental ethics (Palmer et al 2014).

An ecological crisis represents one of the solutions for commercial growth in the economy which is constantly available. Here have been an increase in consumer requests for companies that reduce the adverse environmental effects associated with their related to business procedures. In reaction, businesses must embrace ecological principles as a means ofensure their financial sustainability and also regard the management of the environment as an indicator regarding their "social prosperity." For the purpose of to reduce the destruction which corporate activities, if any of them, had on the natural environment, it is essential for administration to become dedicated to environmental morals by the creation of regulations and implementing them in regular actions. Several research studies indicate which the long term viability of along with group is relying over the ecological consciousness of their humanresources and their respective positions responsibility of particular environmental skills connected with basic operations for organizational programs in their own valuable expansion procedures ( Singh et al 2019).

The intention of the survey's questionnaire is: To offer a summary of the primary areas of passion, advantages, and limitations of the body of studies on learners and instructional methods in the field of environmental education. To identify which were the most major findings from this collection of work for every one of the primary fields that are relevant and assess any drawbacks of these findings in terms of both their empirical foundation and ability to be generalized With the goal to point out problems relating to the natural world, quality, and visibility of fresh studies on the environment. Learning and instruction and highlight fields of concern for further investigation( 2001,Rickinson). The study ecological crisis represents a few of the possibilities of corporate growth which are generally available. Here seems to be a recent increase in public requests from organizations to reduce the adverse ecological effects on their related to business operation. As reaction, businesses must adopt ecological values and a means of ensure their financial sustainability and also regard their management of the environment an indicator to evaluate their "social activities welfare." Managers must be fully motivated to ecological values through developing guidelines and integrating them within everyday events with the aim to decreased the negative environmental effects of the company's activities, whenever any. Many research studies suggest a company's capacity to keep being environmentally friendly depends upon its individual human assets' knowledge regarding sustainability issues and the retention of certain specific environmentally friendly capabilities related to their basic tasks for organizational implementations in their methods of creating values (Singh et al 2019).

## METHEDOLOGY

As a way to assess the sampled population's maturity in knowledge about environmental issues, attitudes, level on treatment, and roles with relation to sustainability and protecting its resources, the research study utilized an organized survey technique for recording data as well as responses. A grand total of the number 138 survey questionnaires had been circulated. A selection at random of teachers present in each of the schools across the both government and private higher primary schools provided their statements. The survey comprises three separate sections: the ethical and educational element, an awareness of environmental issues aspect, plus a mindset toward the condition of the planet component.

## RESULTS AND DISCUSSION

**Environmental Awareness**

Knowing the adverse impacts which our behavior had upon the ecosystem becomes feasible via developing consciousness about environmental issues, and this is essential in community. As a way to maintain the ecosystem to future generations as well as the present, knowledge of encourages ethical behavior, environmentally responsible conduct, plus collective effortsto resolve issues related to the environment.

Environmental pollution has became a big problem ,because a miss- management of municipal waste ,its turns to various problems in the environment ,like air pollution ,causing air borne diseases ,acid rain , green house gases etc .Each individual consume food or water. And exposure of skin to harm-full sunlight may causes serious threat to human health, like skin cancer and others. As per, responses , strongly agreed 21.4% of good response, agreed 42.9% of positive responses, neutral 16.7% average response, disagreed 16.7% medium response and strongly disagree 2.4% were recorded from the Teachers.

It is controversial whether family planning can be made mandatory as a way to help slow up the growth in population. Others argue that it might help in solving the problems caused by a population boom, and others highlight the value of optional, based on rights approaches that takes economical as well as private choices into consideration. Balanced handling of population is often seen as needing public education, access to healthcare, and empowering communities. As response, Strongly agreed 9.5%, agreed 45.2% of positive response, neutral 28.6% of medium response, disagreed 14.3% of average response and strongly disagreed 2.4% response recorded by the Teachers.

Certainly the rapid development of technology and science has enhanced the lives of numerous individuals globally through giving all of a host of positive effects and improvements. Instead it additionally results in a number of harmful impacts and negative impacts on our planet. Probably the primary determinants of damage to the environment are: Industrial pollution, Electronic waste ( E-waste), deforestation, climate change, chemical contamination, loss of biodiversity and others issues. As per responses, strongly agreed 4.8%, agreed 57.1% of positive response, neutral 19.0% of medium response, disagreed 16.7% of average response and strongly disagreed 2.4% responses received from the teachers.

During frequent stops of vehicles in urbanized cities in traffic lights, after delayed in a driving through, or when waiting at a lights. Engine idling has major drawbacks, like wasting fuel and contributing to air contamination, vehicles heating issues and other issues. And spread awareness about these environmental protection duties, because fuels are valuable and limited. And also contribution to global warming due to releasing the green house gases. As per responses, strongly agreed 14.30% of good responses, agreed 33.30% of average responses, neutral 38.10% positive responses, disagreed 11.9% response and strongly disagree 2.40% responses are collected by teachers.

The concept of clearing forests for the reason of providing additional revenue to government departments is challenging and controversial, containing factors related to society, the economy, and the environment. In these issues we are all responsible for forest biodiversity loss, and wildlife animals are enter the cities and make uncomfortable for human living system. And we loss complete forest atmosphere. This concept was spread awareness for each individual for maintain the sustainability for future generations. As per responses, strongly agreed 2.40%, agreed 31.00% of positive responses, neutral 23.80% of medium response, disagreed 28.60% of average response and strongly disagree 14.30% of responses are collected, In concept of forest land, we take serious decision about forest land.

Considering the fact that water occupies 80 percent of the planet's surface, the argument that water pollution isn't an important problem is oversimplified and incorrect. It is true that water occupies an important portion of the Earth's

surface, just ecosystems and life depend on the quantity and purity of that water. The peoples do not know the, what water is covered the earth, there are classified into different types and denoted in percentage, like water is present in the form of ice caps and glaciers, oceans, lakes and other form of water. As per response strongly agreed 4.80%, agreed 38.10% of positive responses, neutral 31.00% of good responses, disagreed 19.00% of average responses and strongly disagreed of 7.10% are feedbacks were recorded.

**Environmental Ethics**

Knowing the ethics of environmental protection is necessary due a variety of explanations, especially knowing that it significantly impacts how individuals conduct each other as well as community in relation to their ecosystem. Implementing ecologically responsible decisions and implementing sustainable practices are crucial for the preservation of natural resources. Listed below are a few tactics that could assist in the preservation of natural resources conservation like, conservation of water, forest, and good atmosphere, and limited usage of natural resource, and nowadays natural resource usage depends on present population note. As per response, 4.30% of not use natural resources, 2.50% of replace worn out resources, 3.50% of restore natural resources and 4.50% of use natural resources wisely, these responses are collected by teachers.

The depletion of ozone layer and the green house effects both are environmental issues, and different reasons for causes in atmosphere by different human activities and technology. Nowadays the depletion of ozone layer and the green house effects leads to global warming and the how many people know about these environmental impact. As per responses 64.30% of too much toxic waste in the atmosphere, 14.30% of atomic explosion, 16.70% of excessive military operations, 2.40% of launching of too many satellites and 2.40% of I don't know, responses are recorded.

Medha patkar is well know as Indian activist, politician, and medha patkar was the initiators of the save the Narmada movement, Narmada Bachao Andolan (NBA). Medha patkar popular leader. As per responses 48.50% of politician, 11.90% of social worker, 50.00% of environmentalist, 33.30% of I don’t know, response are collected.

In this generation, we have was crazy young peoples. They don’t think about environmental responsibility while celebrated some special movements in society. Previous decades are fire the crackers and enjoy, celebrate the festivals time, but nowadays the peoples use fire the fire crackers unnecessary movement like, birthday celebration, VIP entries and special festivals movement. This activity also joined to global warming and etc. The knowledge about this firing and cracker activity. As per response, 57.10% of noise and air pollution, 14.30% of noise and industrial pollution, 4.80% of water and noise pollution, 21.40% of air and water pollution, 2.40% of I don't know, responses are collected.

The Kerala Forum's campaign to stop the reduction of natural resources. It's possible that a project of such a nature developed or became well-known around that date. We are able to offer you more wide knowledge regarding ways community meetings or campaigns, whether in Kerala or in another location, may deal with problems related to the environment. Kerala region has huge biodiversity ecosystem, but human society step into the more income segment to development of nation, so they moderate the forest, like human activity area and wildlife activity area. As per responses, 21.40% of silent valley project , 54.80% of chipko movement , 7.10% narmada andolana, 2.40% of ganga cleaning project and 14.30% of I don't ,know, response are collected.

Solar ovens, occasionally referred to simply as cookers made from sunlight, are machines which roast and boil utilise sunshine as an alternative form of heat. Through transforming direct sunlight into heat, they eliminate the need for standard fuels like oil and wood while cooking food. The following are some essential uses and advantages of solar ovens, environmentally friendly, off –Grid cooking, reduced deforestation and etc. As per responses 40.50% of decreases environmental pollution, 11.90% increases demand for solar ovens, 33.30% increases environmental pollution.7.10% of increases demand for solar energy and 7.10% of I don't know, responses were collected.

**Environmental Education**

Enhancing knowledge about environmental education is necessary for promoting environmentally friendly behaviors and an awareness of environmental responsibility for themselves.

Several individuals and authorities in the subject areas of long-term sustainability and environmental science say that all individuals should place a high premium on conservation of the environment. Because very one use the natural resource in daily life, that's why the every persons conserve and protect the natural resource and environment. As per responses, 26.20% of strongly agreed positive responses, 42.90% of agreed, 19.00% of neutral and 11.90% of disagreed, responses are collected.

Conservation of the environment is an effort which individuals of all ages are capable of contributing to; it is more than anything that professionals do. While adults might have better resources and influence over actions, children and teenagers are still able to contribute a big contribution to building a better tomorrow. These can be some ways citizens may help protect the environment, whichever their age. Schools initiatives, education and awareness to children and teens. As per responses, 9.50% of strongly agreed, 31.00% of agreed, 35.70% of neutral, 21.40% of disagreed and 2.40% of strongly disagreed, responses are recorded

Multidisciplinary instruction and practical relevance are concepts which stand in oppose to the notion of carefully compartmentalizing the teaching of a topic without linking it to the surrounding environment and its various elements. Math, science, language arts, and social studies are just a few of the instances of the subjects which have been classified into specific groups according to conventional educational paradigms. None the less, the beneficial effects of bringing together subjects and presenting them into the wider picture of the environment growing more and more obvious. As per responses 7.10% of strongly agreed, 42.90% of agreed, 33.30% of neutral and 16.70% of disagreed, responses are collected.

A complete approach that helps in the achievement of an in-depth awareness of the environment as well as associated concerns is for integrating environmental issues within the teaching of many subjects related to education. This interdisciplinary strategy recognizes that problems with the environment have complex connections and include multiple disciplines, like social sciences, science, the field of economics and even more. Here several improvements that students observe while environmental factors are presented: interdisciplinary learning, sustainability education. As per responses, 7.10% of strongly agreed, 45.20% of agreed, 33.30% of neutral, 11.90% of disagree and 2.40% of strongly disagreed, responses are collected.

While geography and biology certainly represent essential subjects to understand the basics of and appreciating environmental subjects, it's important that students understand that ecological issues are multidimensional and extend these two academic fields. More general in scope, the field of environmental science combines concepts and ideas from a number of academic, social, and economic areas. As per responses 9.50% of strongly agreed, 38.10% of agreed, 35.70% of neutral, 14.30% of disagreed and 2.40% of strongly disagreed responses are collected.

Considering it increases a sense of environmental responsibility and help learners understand the way various subjects interact with one another, combining environmental principles into various subject areas is usually seen as a helpful approach to education. To avoid any adverse consequences, such as learners losing focus on their main topic, it is essential that this combination be done properly. As per responses 9.50% of strongly agreed, 42.90%, agreed, 26.20% of neutral and 21.40% of disagreed responses are collected.

## CONCLUSION

During nowadays, lifestyle becomes so rapid, the individuals having no spare time to show concern about the surrounding environment. Moreover, the scenario becomes more serious due to the continuing mistreatment and

degradation of earth's resources in the title of industrialization. Materials and resources, which were previously thought to be reusable are currently in risk of growing increasingly restricted to be an outcome of neglect and misuse. The phenomenon becomes especially apparent within the younger generations of community. Humans promote contamination, without caring to or protecting the planet's health. The research was carried out between Government higher primary schools Teachers and Private higher primary schools level Teachers looking to find out more about their views on the environment awareness, education and ethics, and with the objective to evaluate how serious it is of the present situation. The results of the research indicated that Government higher primary schools Teachers and Private higher primary schools level Teachers have grown more aware of and worries about the harm that either intentional or unintentional activity by humans contributes to the ecosystem. Their responses to the questionnaire's topics highlighted an excellent aspect about their awareness of the environment. That is why schools, educational institutions must provide environmental ethics, awareness and education on the threats to health related to pollution from the environment, along with a number of events. These will allow it less difficult for recruits more and more Teachers and other people to join collaboratively to protect the globe's environmental sustainability.

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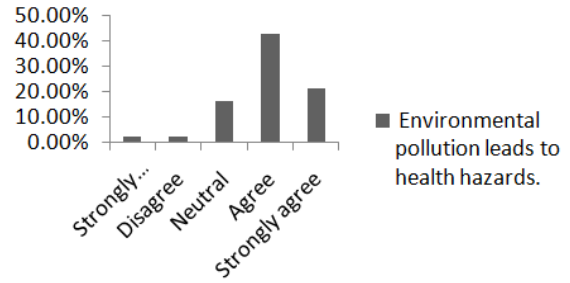


**Fig.1- Responses regarding Environmental pollution leads to health hazards.**

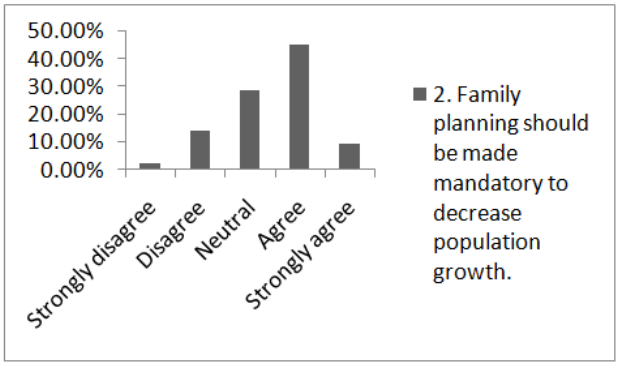


**Fig.2- Responses regarding family planning was mandatory to decrease population growth.**

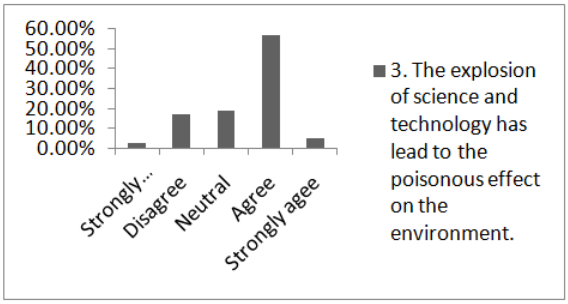


**Fig.3- Responses regarding the explosion of science and technology has lead to the poisonous effect on the environment.**

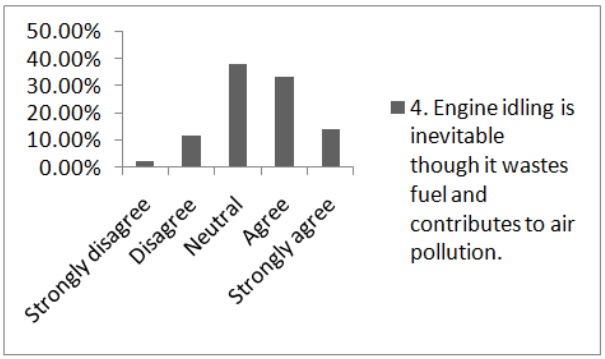


**Fig.4- Responses regarding engine idling is inevitable though it wastes fuel and contributes to air pollution**

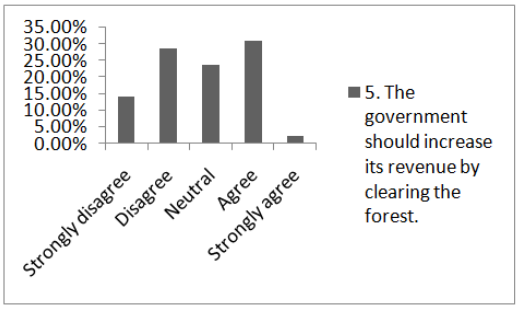


**Fig.5- Responses regarding the government should increase its revenue by clearing the forest.**

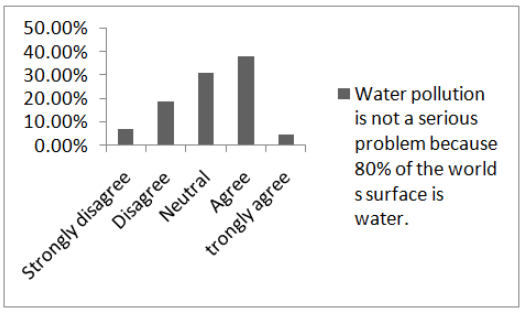


**Fig .6- Response regarding the water pollution is not a serious problem because 80% of the world s surface is water.**

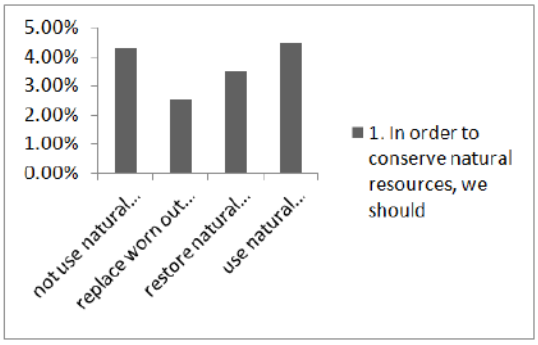


**Fig.7- Responses regarding In order to conserve natural resources, we should**

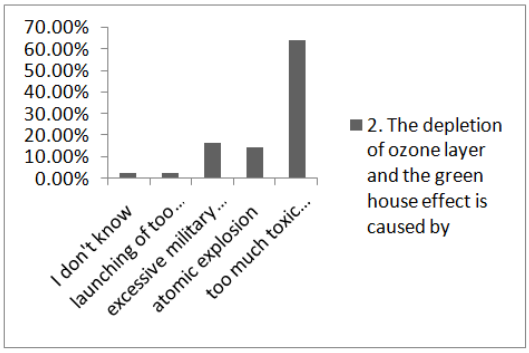


**Fig.8- Responses regarding the depletion of ozone layer and the green house effect is caused by**

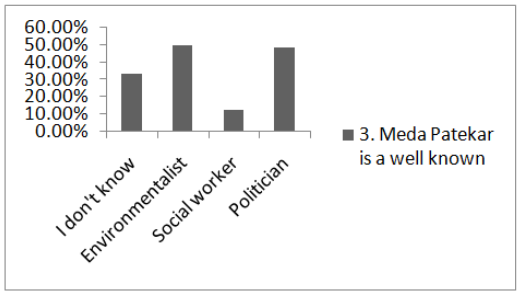


**Fig.9- Responses regarding, Medha Patkar is a well known**

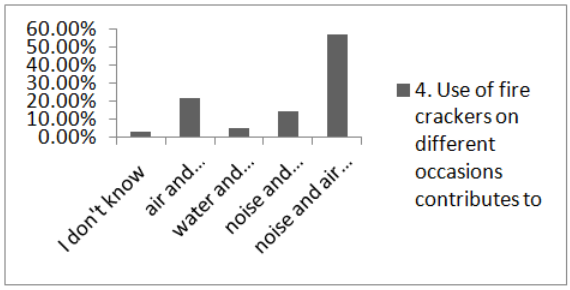


**Fig.10- Responses regarding , Medha Patkar is a well known**

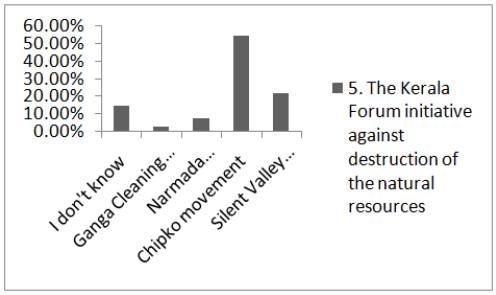


**Fig.11- Response regarding the Kerala Forum initiative against destruction of the natural resources**

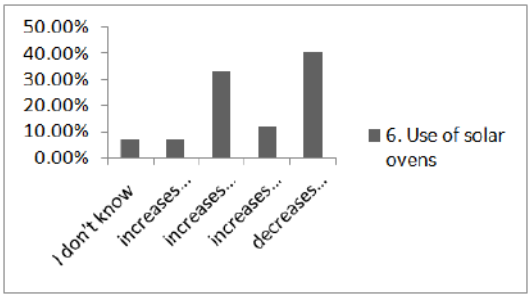


**Fig.12- Responses regarding Use of solar ovens**

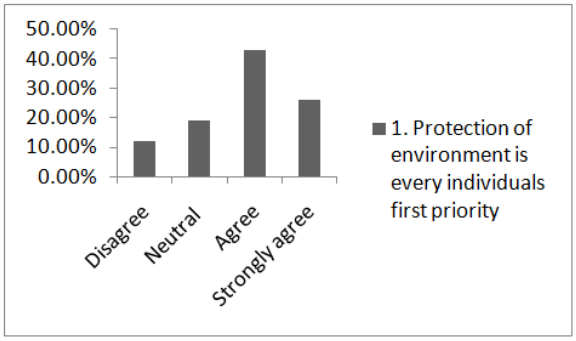


**Fig.13- Responses regarding protection of environment is every individuals first priority.**

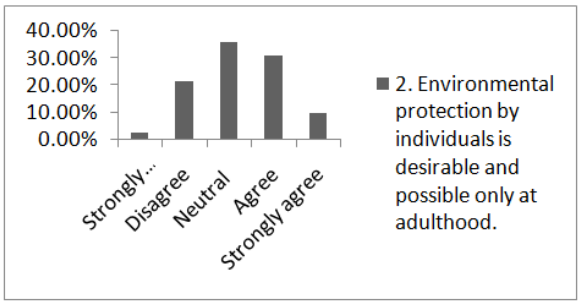


**Fig.14- Responses regarding environmental protection by individuals is desirable and possible only at adulthood.**

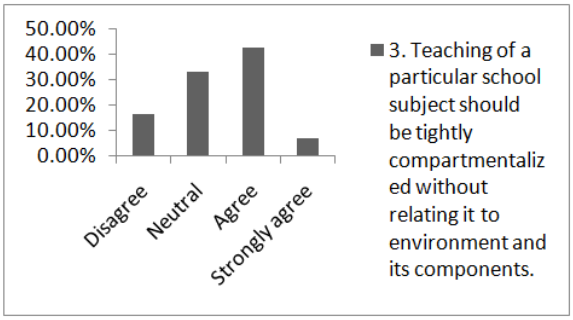


**Fig.15- Responses regarding teaching of a particular school subject should be tightly compartmentalized without relating it to environment and its components.**

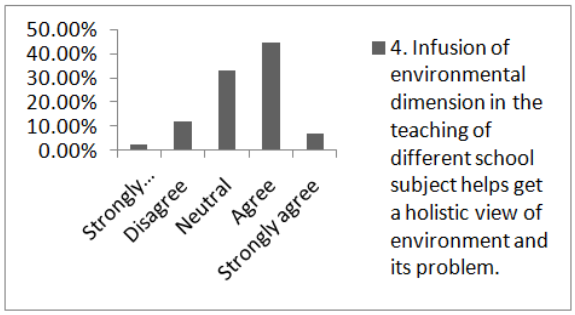


**Fig.16-Response regarding infusion of environmental dimension in the teaching of different school subject helps get a holistic view of environment and its problem.**

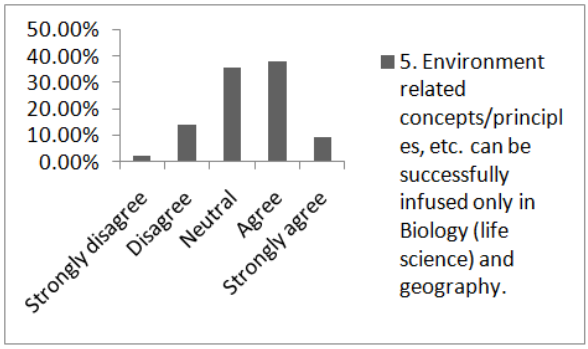


**Fig.17- Responses regarding environment related concepts/principles, etc. can be successfully infused only in Biology (life science) and geography.**

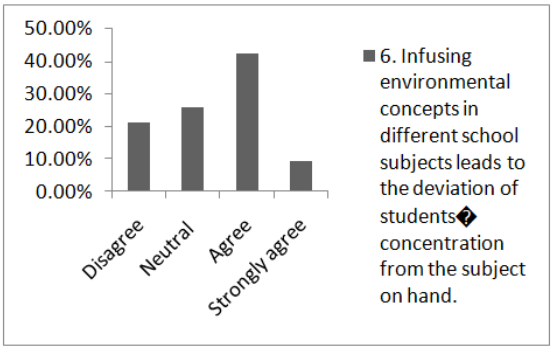


**Fig.18- Responses regarding infusing environmental concepts in different school subjects leads to the deviation of student's concentration from the subject on hand.**

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**RESEARCH ARTICLE**

# Morphometric Analysis of Krishnarajapete Watershed using Geospatial Techniques, Mandya District, Karnataka, India

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**ABSTRACT**

The morphometric properties of a watershed can be estimated using Remote sensing (RS) and Geographic information system (GIS) approaches. The watershed was divided into 11 sub-watersheds (SWs), which are then ranked according to morphometric characteristics. For each sub-watershed, morphometric features such as aerial and linear aspects have been evaluated to determine a preferred ranking. The drainage basin is characterized by a dendritic to subdendritic drainage pattern, according to morphometric analysis. The analysis also showed that the total number and length of stream segments is maximum in first-order streams and decreases as the stream order increases. Theelongation ratio is less than 0.60 for all 11 sub-watersheds, indicating that it has a moderately sloped surface and an elongated shape.Low drainage density leads to coarse drainage texture in the study area. Flood flows easier in the elongated basin so 10 sub-watersheds except 1 sub-watershed show elongation of the shape of the basin.

**Keywords:** Morphometry, Sub-Watershed,Geographic Information System, Lithology.

## INTRODUCTION

The measuring and quantitative analysis of the landforms' dimensions, shapes, and configurations on Earth is known as morphometry[1,2]. To ensure long-term growth, avoid soil erosion, and save water, watersheds must be studied. Remote sensing and geographic information system (GIS) analysis are effective methods for examining the hydraulic process in the river basin[3].Any hydrological inquiry, including pedology, environmental evaluation, groundwater management, and groundwater potential assessment, has to consider the drainage basin analysis. Hydrologists and geomorphologists have determined that certain relationships between runoff characteristics and drainage basin systems' geographic and geomorphic features are crucial. The physiographic features of drainage basins, such as size,

shape, slope of the drainage area, drainage density, size and length of the contributors, etc., can be connected with several significant hydrologic phenomena[4].The Watershed is the best unit for managing natural resources and promoting sustainable development[5].To be able to properly define and analyze drainage basins, the most recent Remote Sensing and GIS techniques must be integrated[6].Scientific studies indicate that morphometric characteristics of a river basin are important in determining which sub-watersheds should be prioritized. Water flowing into and out of the basin will deposit and accumulate contaminants, fertilizers, and sediments [7,8]. The onsite and offsite ecology of the river basin may be significantly impacted by them. Therefore, researching the drainage basin process may contribute to a deeper comprehension of the hydrologic cycle and the movement of water. To achieve sustainable land and water resource use and lessen the effects of growing pollution, watershed management must be put into practice [9-11].These days, geographic information system (GIS) approaches are employed.

For evaluating the different topography and morphometric characteristics of the watersheds and drainage basins, since they offer a versatile setting and an effective instrument for the handling and examination of geographical data. Since there hasn't been a thorough morphometric investigation of the area done before, the primary goal of the current study is to use a Geographic Information System (GIS) to analyze the linear and areal morphometric characteristics of the watershed. The management of the region's water resources and other natural resources is aided by this study's knowledge of the many geo-hydrological features of the watershed[12].The most pertinent quantitative morphometric features have been selected and used for the current investigation. Three groups of morphometric features, such as linear, relief, and areal aspects, can be distinguished. Since they have a connection to flow, runoff, and soil erosion threats, they have been used to prioritize more vulnerable sub-watersheds[13, 14].The goal of the current study is to rank the sub-watersheds according to their individual morphometric properties within the watershed of Krishnarajapete.

## MATERIALS AND METHODS

**Study area**

Krishnarajapete (KRP), also referred to as K.R. Pete is a taluk and municipality in the Mandya District of the Indian state of Karnataka. It has an area of 896 square kilometres and is located between longitude 76°19' 47" E and latitude 12°27'04" N. It is included in Toposheets numbers are 57D/5, 57D/6, 57D/9, 57D/12, and 57C/16, which were published by the Survey of India (SOI) and are depicted in Fig. 1. With the use of software NRSC and hydrosheds, the Krishnarajapete watershed has been divided into 11 sub-watersheds, as seen in Figure 2.

**Lithology**

As per the bhukosh information the extracted area shows Amphibolite, biotite schist, calc gneiss, dolerite, dunite, fuchsite quartzite, granite gneiss, granitoid, kyanite-staurolite-mica schist, leuco gneiss, metabasalt, meta ultramafite, pegmatite, quartzite, and quartz reef shown in figure 3. All these rocks are metamorphic and show excellent fracture and lineament zones.

**Drainage**

The area shows a good dendritic to sub-dendritic pattern of drainage pattern. The stream that follows this path has been divided into stream orders. The Krishnarajapete watershed shows 5 orders shown in Figures 4a and 4b. Some of the orders were absent in the subwatershed. The 11 individual sub-watersheds in a Krishnarajapete watershed are shown in Figure 5. Morphometry is the study of how water and sediment move across a basin and how the geometries of the stream and basin networks relate to this movement. Measurements of the drainage network's linear aspects, drainage basin's areal aspects, relief (gradient) aspects of the channel network, and contributing ground slopes are necessary for a systematic description of a drainage basin's geometry and its stream channel (Strahler, 1964). Using the mathematical formulas listed in (Table 1), the morphometric analysis for the parameters in the study

is conducted concerning stream order, stream length, bifurcation ratio, stream length ratio, basin length, drainage density, stream frequency, elongation ratio, circularity ratio, and form factor, etc.

**Stream Order (Sμ)**

There are 5 stream orders in the entire Krishnarajapete district. There are a total of 64 streams are present. Individual streams in each sub-watershed are shown in Table 2. Order 1 refers to the tiniest fingertip tributaries. A channel segment of order 2 is produced where two first-order channels unite, a segment of order 3 is generated where two order-2 channels combine, and so on. Therefore, the stream segment of the greatest order is the mainstream, through which all water and sediment discharge travels[15].

**Stream length (Lμ)**

The length of the stream indicates how the segments have developed chronologically, including any tectonic disturbances that may have occurred in between.Horton's principle states that there is a negative correlation between the order and the number of streams[16]. Out of 11 sub-watersheds the highest length of all order streams found in WS-9 is about 36.83 km and the lowest in WS-11 of about 2.11km as shown in Table 2.

**Mean stream length (Lsm)**

The average stream length provides insight into the typical dimensions of drainage network elements and the surfaces that contribute to them[15]. In the study mean stream length varies from 0.14 km to 10.52 km.

**Bifurcation Ratio (Rb)**

The bifurcation ratio, which is the ratio of the total number of stream segments of one order to that of the next higher order in a drainage basin, is connected to the branching pattern of a drainage network[17]. The lower value and absence of the order value indicate less number of structural disturbances. The range from 0.25 to 2 is shown in Table 3.

**Stream Length Ratio (RL)**

The mean stream length of a particular order divided by the mean stream length of the next lower order is known as the stream length ratio, and it has a significant impact on the basin's erosion stage, surface flow, and discharge[18]. The values vary from 0.05 to 17.19, it completely depends on the area slope.

**Mean Bifurcation Ratio ($R_{bm}$)**

The average of all bifurcation values is the mean bifurcation ratio, which was in the particular area is 1.92.

**Basin Perimeter (P)**

The outside line enclosing the drainage basin's area is known as the basin perimeter. It may be used as a gauge for the size and form of basins as it is measured along the lines separating them[19].

**Basin length (Lb)**

From the catchment to the point of confluence, it is the basin's longest length. The longest basin length in WS-9 is about 22.79 km and the lowest is 2.13 km in WS-11 as shown in Table 4.

**Length of overland flow (Lg)**

Percolation through the soil and infiltration (exfiltration), which both vary in time and place, have a major impact on overland flow[20]. The surface runoff varies from 0.56 to 1.06 as shown in Table 4.

**Basin Area**

A gathering area from which water would be directed toward a stream or river is referred to as a drainage region. The ridge that divides water flowing in opposing directions defines the area's border. The least area of 17.15 $km^2$ in WS-4 and the highest of 174.24 $km^2$ in WS-5 is shown in Table 4.

**Drainage density (Dd)**
It is known that drainage density plays a significant role in influencing how long water takes to move. In study area varies from 0.16 to 0.30 shown in Table 4. The fact that the research area's drainage density is low, it is evident that the area has medium relief, moderately dense plant cover, and permeable subsurface[21].

**Drainage frequency (Fs)**
Flooding is more common in basins with high drainage and stream frequency because higher drainage densities and stream frequencies cause quicker runoff[22]. In the study area drainage frequency was very low so more likely to flood 0.04 to 0.13 shown in Table 4.

**Drainage Texture (Dt)**
Less than two drainage density denotes a very coarse drainage texture, between two and four, a moderate drainage texture, between four and six, a fine drainage texture, and more than eight, a very fine drainage texture[23]. In all 11 sub-watersheds, the drainage texture is coarse shown in Table 4.

**Form factor ratio (Rf)**
A smoother flow peak for a longer period is predicted by the extended basin with a low form factor. Compared to circular basins, such elongated basins are easier to regulate flood flows[24]. In the study area form factor is high only in circular shape WS-11 shown in Table 4.

**Elongation ratio (Re)**
It is a highly important index in the analysis of basin shape that provides information about a drainage basin's hydrological characteristics. Low-relief locations are often represented by values close to 1. In the study area, all values except WS-4 and WS-11 show low relief as shown in Table 4.

**Circularity ratio (Rc)**
It is represented as the ratio of the basin area to the area of a circle with the same perimeter as the basin and is used as a quantitative metric to visualize the basin's form. It varies from 0.23 to 0.60, which shows the medium to low relief and approximate elongation of the drainage basin.

## CONCLUSION

The watershed is categorized as a 5-orderstream based on the drainage orders. The average Rb suggests that geological formations have little effect on the drainage pattern. The most helpful criteria for the morphometric categorization of drainage basins are drainage density (Dd) and stream frequency (Fs), which unquestionably regulate the runoff pattern, sediment output, and other hydrological parameters of the drainage basin. The underlying strata's porous character is shown by the basin's Drainage density.The dendritic drainage system that dominates the watershed helps to explain several topographical features, such as runoff and infiltration rate. Regions for surface-water accumulation and recharge-related activities that may be put into practice for watershed management are highlighted by the assessed parameters in this study.

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**Conflict of interest**

There is no conflict of interest among authors.

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**Table 1. Morphometry parameters formulas**

| S. No. | Parameters | Formula | Reference |
|---|---|---|---|
| 1 | | Linear Morphometric parameters | |
| 1.1 | Stream Order (Sμ) | Hierarchical rank | Strahler (1964) |
| 1.2 | Bifurcation Ratio ($R_b$) | $R_b = N\mu / N\mu + 1$<br>Where, Rb = Bifurcation ratio,<br>Nμ = No. of stream segments of a given order and<br>Nμ +1= No. of stream segments of next higher order. | Schumn (1956) |
| 1.3 | Mean Bifurcation Ratio ($R_{bm}$) | $R_{bm}$ = Average of bifurcation ratios of all orders | Strahler (1964) |
| 1.4 | Stream Length (Lμ) | Length of the stream (kilometers) | Horton (1945) |
| 1.5 | Mean Stream Length ($L_{sm}$) | $L_{sm} = L\mu/N\mu$<br>Where, Lμ = Total stream length of order 'μ'<br>Nμ = Total no. of stream segments of order 'μ' | Strahler (1964) |
| 1.6 | Stream Length Ratio ($R_L$) | $R_L = L_{sm} / L_{sm}-1$<br>Where, $L_{sm}$=Mean stream length of a given order and<br>$L_{sm}$-1= Mean stream length of next lower order | Horton (1945) |
| 1.7 | Length of Overland Flow ($L_g$) | $L_g$=1/2D Km<br>Where, D=Drainage density (Km/Km$^2$) | Horton (1945) |
| 1.8 | Basin Perimeter (P) | P=Outer boundary of drainage basin measured in kilometers. | Schumm (1956) |
| 1.9 | Basin Length ($L_b$) | $L_b=1.312*A^{0.568}$ | Gregory and Walling (1973) |
| 1.10 | Fitness Ratio ($R_f$) | $R_f = C_L/P$<br>Where, $C_L$= Main channel length (Kms) and<br>P= Basin perimeter (Kms) | Melton (1957) |
| 1.11 | Wandering Ratio ($R_w$) | $R_w = C_L/L_v$<br>Where, $C_L$ = Main channel length (Kms) and<br>$L_v$ = Valley length (Kms) | Smart and Surkan (1967) |
| 1.12 | Standard Sinuosity Index (SSI) | $SSI = C_L/L_v$<br>Where, $C_L$ = Channel length (Kms) and<br>$L_v$ = Valley length (Kms) | Muller (1968) |
| 2 | | Areal Morphometric parameters | |
| 2.1 | Basin Area (A) | Area from which water drains to a common stream and boundary determined by opposite ridges. | Strahler (1969) |
| 2.2 | Drainage Density ($D_d$) | $D_d = L\mu/A$<br>Where, Dd = Drainage density (Km/Km$^2$)<br>Lμ = Total stream length of all orders and<br>A = Area of the basin (Km$^2$). | Horton (1932) |
| 2.3 | Drainage Frequency ($F_s$) | $F_s = N\mu/A$<br>Where, $F_s$ = Drainage frequency.<br>Nμ = Total no. of streams of all orders and<br>A = Area of the basin (Km$^2$). | Horton (1932) |
| 2.4 | Drainage Texture ($D_t$) | $D_t = N\mu /P$<br>Where, Nμ = No. of streams in a given order and P = Perimeter (Kms) | Smith (1950) & Horton (1945) |
| 2.5 | Form Factor Ratio ($R_f$) | $R_f = A/L_b^2$<br>Where, A = Area of the basin and<br>$L_b$ = (Maximum) basin length | Horton (1932) |
| 2.6 | Elongation Ratio ($R_e$) | $R_e = \sqrt{A} / \pi / L_b$<br>Where, A= Area of the Basin (Km$^2$)<br>$L_b$=Maximum Basin length (Km) | Schumm (1956) |
| 2.7 | Circularity Ratio ($R_c$) | $R_c = 4\pi A/ P^2$<br>Where, A = Basin Area (Km$^2$) and<br>P= Perimeter of the basin (Km)<br>Or $R_c = A/ A_c$<br>Where, A = Basin Area (Km$^2$) and<br>$A_c$ = area of a circle having the same perimeter as the basin | Miller (1953) |

**Table 2. Stream orders, number, length(km), and Mean stream length.**

| Sub-watershed numbers | Stream orders number(Sμ) | Streams in numbers | Total stream In numbers ΣSu | Stream length (Lμ) | Σ (Lμ) | Mean stream length (Lsm) |
|---|---|---|---|---|---|---|
| **WS-1** | 1st | 1 | 1 | 4.61 | 4.61 | 4.61 |
| **WS-2** | 1st | 2 | 7 | 0.28 | 13.55 | 0.14 |
| | 4th | 1 | | 3.11 | | 3.11 |
| | 5th | 4 | | 10.16 | | 2.54 |
| **WS-3** | 1st | 4 | 11 | 11.61 | 31.48 | 2.90 |
| | 2nd | 2 | | 9.15 | | 4.58 |
| | 3rd | 1 | | 0.49 | | 0.49 |
| | 5th | 4 | | 10.23 | | 2.56 |
| **WS-4** | 4th | 1 | 1 | 3.38 | 3.38 | 3.38 |
| **WS-5** | 1st | 5 | 13 | 11.96 | 39.58 | 2.39 |
| | 2nd | 1 | | 1.75 | | 1.75 |
| | 3rd | 1 | | 0.23 | | 0.23 |
| | 5th | 6 | | 25.64 | | 4.27 |
| **WS-6** | 2nd | 2 | 3 | 0.54 | 9.82 | 0.27 |
| | 3rd | 1 | | 9.28 | | 9.28 |
| **WS-7** | 1st | 3 | 5 | 16.23 | 26.03 | 5.41 |
| | 2nd | 2 | | 9.80 | | 4.90 |
| **WS-8** | 1st | 3 | 5 | 10.06 | 23.55 | 3.35 |
| | 2nd | 2 | | 13.49 | | 6.75 |
| **WS-9** | 1st | 4 | 7 | 11.36 | 36.83 | 2.84 |
| | 2nd | 2 | | 21.04 | | 10.52 |
| | 3rd | 1 | | 4.43 | | 4.43 |
| **WS-10** | 1st | 4 | 9 | 7.87 | 24.75 | 1.97 |
| | 5th | 5 | | 16.88 | | 3.38 |
| **WS-11** | 1st | 1 | 2 | 2.10 | 2.88 | 2.10 |
| | 3rd | 1 | | 0.78 | | 0.78 |

**Table 3 Stream length ratio and Bifurcation Ratio**

| Sub-watershed numbers | Stream Length Ratio (RL) | | | | Bifurcation ratio (Rb) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2nd/1st | 3rd/2nd | 4th/3rd | 5th/4th | 1st/2nd | 2nd/3rd | 3rd/4th | 4th/5th |
| **WS-1** | - | - | - | - | - | - | - | - |
| **WS-2** | - | - | - | 3.26 | - | - | - | 0.25 |
| **WS-3** | 0.79 | 0.05 | - | - | 2 | 2 | - | - |
| **WS-4** | - | - | - | - | - | - | - | - |
| **WS-5** | 0.15 | 0.13 | - | - | 5 | 1 | - | - |
| **WS-6** | - | 17.19 | - | - | - | 2 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **WS-7** | 0.60 | - | - | - | 1.5 | - | - | - |
| **WS-8** | 1.34 | - | - | - | 1.5 | - | - | - |
| **WS-9** | 1.85 | 0.21 | - | - | 2 | 2 | - | |
| **WS-10** | - | - | - | - | - | - | - | - |
| **WS-11** | - | - | - | - | - | - | - | - |

**Table 4. Perimeter, Basin Length, Length of Overland Flow, Basin Area, Drainage Density, Drainage/ stream Frequency, Drainage Texture, Form Factor Ratio, Elongation Ratio, and Circularity Ratio**

| Sub-watershed Number | Perimeter (P) | Basin Length (Lb) | Length of Overland Flow (Lg) | Basin Area (A) | Drainage Density (Dd) | Drainage/ stream Frequency (Fs) | Drainage Texture (Dt) | Form Factor Ratio (Rf) | Elongation Ratio (Re) | Circularity Ratio (Rc) |
|---|---|---|---|---|---|---|---|---|---|---|
| **WS-1** | 33.72 | 7.92 | 0.70 | 23.02 | 0.20 | 0.04 | 0.03 | 0.37 | 0.68 | 0.25 |
| **WS-2** | 42.92 | 9.64 | 0.85 | 55.65 | 0.24 | 0.13 | 0.16 | 0.60 | 0.87 | 0.38 |
| **WS-3** | 55.10 | 19.19 | 0.89 | 123.12 | 0.26 | 0.09 | 0.20 | 0.33 | 0.65 | 0.51 |
| **WS-4** | 22.67 | 2.97 | 0.69 | 17.15 | 0.20 | 0.06 | 0.04 | 1.94 | 1.57 | 0.42 |
| **WS-5** | 60.50 | 16.35 | 0.80 | 174.24 | 0.23 | 0.07 | 0.21 | 0.65 | 0.91 | 0.60 |
| **WS-6** | 30.98 | 9.16 | 0.98 | 35.05 | 0.28 | 0.09 | 0.10 | 0.42 | 0.73 | 0.46 |
| **WS-7** | 49.76 | 14.15 | 1.02 | 89.30 | 0.29 | 0.06 | 0.10 | 0.45 | 0.75 | 0.45 |
| **WS-8** | 55.68 | 17.52 | 0.68 | 121.16 | 0.19 | 0.04 | 0.09 | 0.39 | 0.71 | 0.49 |
| **WS-9** | 89.49 | 22.79 | 0.88 | 146.24 | 0.25 | 0.05 | 0.08 | 0.28 | 0.60 | 0.23 |
| **WS-10** | 45.64 | 12.61 | 1.06 | 81.91 | 0.30 | 0.11 | 0.20 | 0.52 | 0.81 | 0.49 |
| **WS-11** | 29.61 | 2.13 | 0.56 | 18.03 | 0.16 | 0.11 | 0.07 | 3.97 | 2.25 | 0.26 |

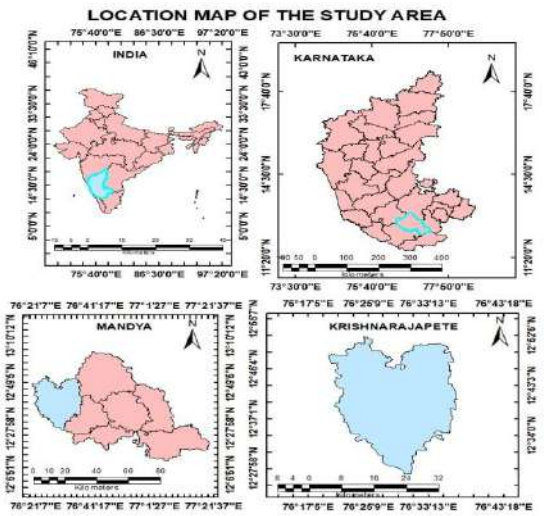


**Fig. 1. Location Map of the Study Area**

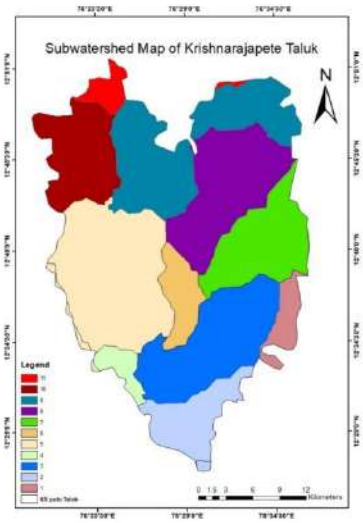


**Fig. 2. Sub-watershed map of Krishnarajapete**

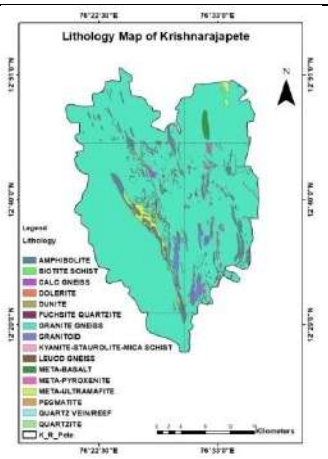


**Fig. 3. Lithology map of Krishnarajapete.**

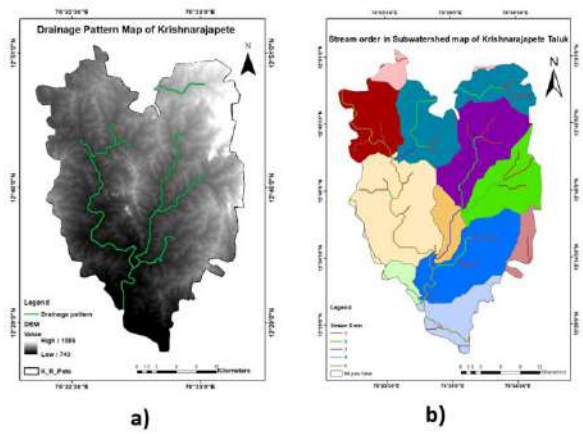


**Fig. 4. GIS map of a) Drainage map, and b) Stream order map**

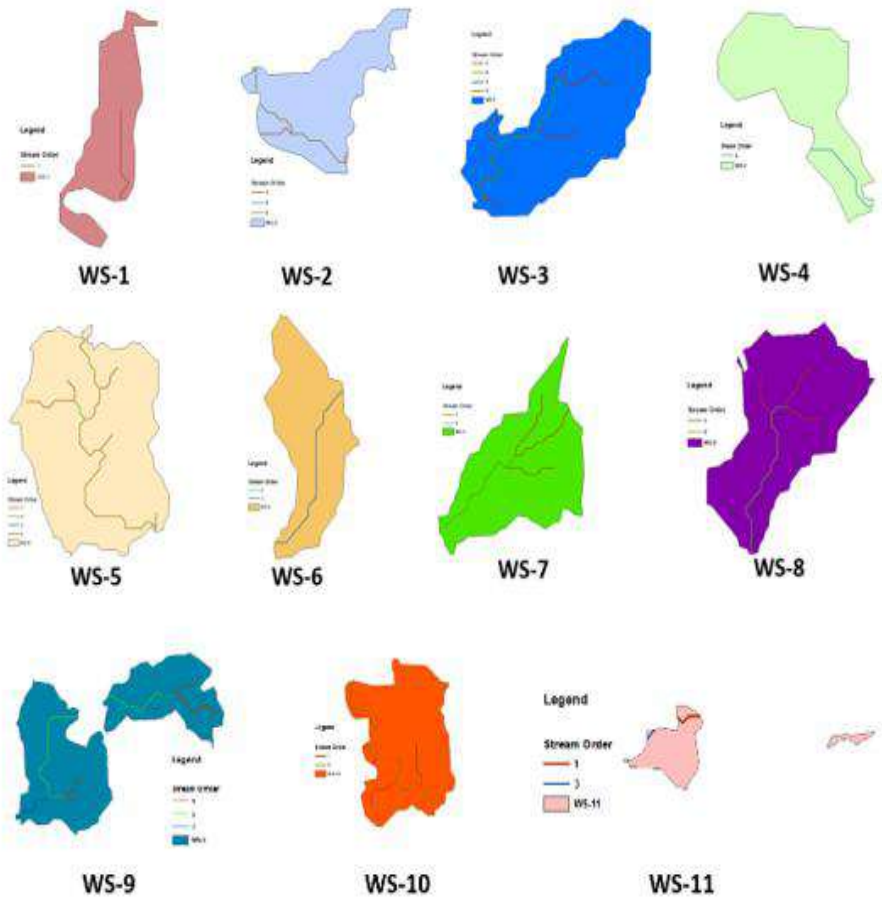


**Fig. 5. The eleven individual sub-watershed map**

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**RESEARCH ARTICLE**

# Design and Implementation of a Framework for IoT- based Smart Attendance Management System

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## ABSTRACT

This research paper outlines an innovative attendance management system leveraging IoT, AWS, and an RFID module with an Arduino Uno board. This system aims to enhance accuracy and efficiency in attendance tracking, overcoming the limitations of traditional methods. Comprising both hardware and software elements, the hardware integrates an RFID module connected to an Arduino Uno board for capturing attendance data. The software, developed using Python Django and hosted on AWS, is responsible for processing and storing attendance information. This architecture offers real-time attendance monitoring and reporting, accessible through web or mobile applications from any location. Tested in a real-world scenario using RFID-enabled tags or cards for attendance tracking, the implemented system demonstrated superior accuracy and efficiency compared to conventional attendance management systems. The proposed solution provides a reliable, cost-effective approach for attendance management, applicable across various organizational settings. By facilitating real-time tracking and

reporting through web and mobile interfaces, the system offers an effective alternative to traditional attendance management methods, promising heightened accuracy and efficiency.

**Keywords:** RFID, AWS, IoT, hardware, Python, Arduino.

## INTRODUCTION

An efficient method of managing attendance is an essential component of the successful operation of any organization, but this is especially true in the fields of education and business. Conventional methods of documenting attendance rely on human procedures, which are prone to mistakes and can result in inefficiencies. Modern technologies provide more accurate and time-saving alternatives. The advent of the Internet of Things (IoT) and cloud computing has brought about a substantial shift in attendance management. As a result, the accuracy and efficacy of the aforementioned systems have been significantly improved.

The investigation of the incorporation of Internet of Things (IoT), Amazon Web Services (AWS), and Radio Frequency Identification (RFID) technologies within the framework of Python Django and Arduino Uno is the major purpose of our research. This integration is intended to provide an innovative strategy for the administration of attendance systems. The purpose of this project is to solve the constraints of existing systems while also automating attendance procedures. This investigation presents a novel strategy for managing attendance, with the goals of improving accuracy, productivity, and cost-effectiveness. The system may be broken down into its two basic parts, which are the hardware and the software. The hardware configuration for the purpose of collecting attendance data comprises of an RFID module that is coupled to an Arduino Uno board. This board is used for the purpose of collecting data. On the Amazon Web Services (AWS) Cloud platform is where the program that was built with the Python Django framework is currently running. The storage and processing of attendance data is the major responsibility that it is tasked with handling. The aforementioned architectural design makes it possible to record and report attendance in real time, information that can be accessed easily through either web-based or mobile apps thanks to the design's convenience.

The automation of attendance tracking procedures is a direct outcome of the incorporation of Internet of Things (IoT) technology into attendance management systems. Figure. 1. shows the whole architecture of the hardware component. Because human data entry is no longer necessary as a result of this automation, the possibility of making a mistake has been significantly reduced. RFID modules are able to scan RFID tags or user cards, which makes it easier to gather data on attendance. These modules may be used in conjunction with RFID readers. After the data has been gathered, these modules will send it to the AWS Cloud, where it will be processed and stored. Cloud computing from Amazon Web Services (AWS) provides a solution for managing attendance that is scalable, reliable, and cost-effective. These are the three main characteristics of the solution. Because Amazon Web Services (AWS) offers a variety of services that include storage, processing, and administration tools, it is a particularly excellent platform for hosting attendance management systems. These services may be found here. In addition, Amazon Web Services makes it easier for users to access their accounts by enabling seamless system usage from any place provided they have an internet connection.

Python Django, a web development framework that is widely used and well-known for its capacity to speed up the construction of online applications, was used in the implementation of the software component of our suggested system. This allowed us to realize the system more quickly. Python is an excellent choice as a programming language for the development of software components that are employed in attendance management systems because of its longevity, versatility, and ease of learning. These qualities make Python an ideal choice. Django is a web application framework that provides a complete set of tools that can be used to build web applications very efficiently. These

tools cover fundamental aspects of web application development such as authentication, data modeling, and data security. When it comes to the context of attendance management systems, these characteristics are very crucial. The architectural model that is being discussed in this article has already been implemented and put through rigorous testing in real-world settings, making use of RFID attendance badges or maps as the data source. These studies have proved that the model possesses superior levels of accuracy and efficiency when compared to other attendance management systems that are currently available.Figure. 2. shows the whole architecture of the hardware component. The architecture that we have built provides a dependable and cost-effective solution for monitoring attendance, and it is applicable to a very wide variety of businesses.

**Methods of Experimentation and/or Methodology**
**The Methodical Approach**
**Identification of the Problem**

The first stage entails the identification of limitations that are associated with conventional attendance management systems as well as the potential improvements that may be achieved via the integration of Internet of Things (IoT) and cloud computing technologies, which will ultimately result in an increase in the system's accuracy and efficiency. This will be done in order to set the stage for the subsequent stages. In order to get a more in-depth comprehension of the subject at hand, the purpose of the current investigation is to carry out an exhaustive analysis of the previously published research on the matter. An exhaustive investigation into the existing attendance management systems and the inherent limitations of such systems is being carried out. This requires doing an in-depth analysis of Internet of Things (IoT) and cloud computing technologies to determine which tools and platforms are most suited for the proposed system.

The creation of a system is going to be covered in this round of conversation. The attendance management system has been designed with an emphasis on architecture, with components drawn from both the hardware and software realms. An RFID module that is attached to an Arduino Uno board is the component of the hardware that is used to collect attendance data. This board is used for the purpose of acquiring information about attendees. The software component was developed using the Python Django framework, and it is now hosted on the cloud infrastructure provided by Amazon Web Services (AWS). The storing and processing of attendance records is the major purpose of the system.

**System Implementation**
After the proposed system has been designed, it is next put into action and assessed in a real-world environment employing an RFID-enabled tag or card for the purpose of registering attendance. An investigation is carried out on the data on attendance that was gleaned from the proposed system. To evaluate whether or not improvements have been made in terms of accuracy and efficiency, the findings that were collected are compared with those of the conventional attendance management system.

**Consideration and Concluding Thoughts**
An evaluation of the effectiveness of the attendance management system that is discussed in this research is carried out by carefully examining the data. A judgment may be drawn on the effectiveness of the system and the potential value it possesses in a wide variety of organizational settings.

**The Accumulation and Evaluation of Information**
For determining who attended a certain event, the gathering of data makes use of an RFID module that is fastened to an Arduino Uno board. After the data has been obtained, it is then evaluated by the software module that was written utilizing the Python Django framework and then placed on the cloud platform that is provided by Amazon Web Services (AWS). The current technique makes it possible to monitor and document attendance in real time. This information may be accessible easily through a web application or a mobile application, depending on your preference. The information that has been compiled includes attendance records for individual students or

employees. These records include the individuals' names, as well as the date, the time, and any other significant information.

**Analysis of the Data**

The data that has been acquired is analyzed using a variety of statistical methods, including but not limited to measurements such as mean, median, standard deviation, and other appropriate approaches. This analysis is performed on the data that has been gathered. In the research article, the analytic results are presented with graphical representations for the purpose of providing a thorough understanding of the system's effectiveness and efficiency. The following are some examples of formulas used for the purpose of data analysis:

The mean is determined by taking the total number of observations (n) and dividing that figure by the sum of all the values (xi). When there are an odd number of values in a dataset, the method for computing the median is given by dividing the total number of values plus one by the second value in the dataset.
The following is the formula that should be used when computing the median of a collection of data that has an even number of values (n): The median is determined by taking the average of the value that is (n / 2)nd and the value that is ((n / 2) + 1)th.

The formula for calculating the standard deviation, which is denoted by the letter s, is s = sqrt((1/n) * (xi - x)2, where the letter s denotes the standard deviation. The value n denotes the complete number of observations contained inside the dataset. The value shown by the symbol is the total of the values under consideration.
Individual data points are represented by the variable xi in this sentence.
In the following, we will refer to the arithmetic mean of the data collection as x.
The evaluation takes into account a wide variety of factors, including the system's accuracy, efficiency, reliability, and cost-effectiveness.

**Limitations and Restrictions**

The attendance management system that is being offered was designed with a specific hardware and software architecture, which may not be appropriate in all contexts. This is one of the limitations of the system. The performance of the system is contingent on the existence of the network and the cloud infrastructure as well as their level of consistency, which has the potential to impair its reliability. The software component is composed of three main modules: the AWS EC2, the AWS API Gateway, and the Python Django application refer Figure. 3. The accuracy of attendance records is dependent on the efficient operation of the RFID module and the uniformity of the tag or card that is used. The accuracy of attendance records is dependent on the uniformity of the tag or card that is used. The size of the study's sample was restricted, which may have hindered its capacity to capture all of the many organizational or scenario variations that are feasible.

Limitations: The major goal of this study was to construct and analyze the efficacy of an attendance management system that includes Internet of Things (IoT), Amazon Web Services (AWS), and a Radio Frequency Identification (RFID) module. This purpose was accomplished by conducting this research. Python Django and a board designed for Arduino Uno were utilized during the process of putting this system into operation. Due to the fact that the study's scope was restricted to the education and corporate sectors, it is possible that the recommended strategy will not be applicable to other fields to the same extent. Because this research was based on a single case study, the data collection and analysis were carried out using that methodology. Because of this, it is possible that the findings cannot be generalized to other contexts or organizations. The study did not include an investigation of the moral and legal implications of utilizing RFID technology for attendance management. This was omitted from the scope of the research.

**The Suggested Architecture for the System**

**Prerequisites for the System**

The hardware is:

- Internet connectivity for the Arduino UNO R3 board • RFID-enabled tags or cards • RFID module
- Python, the Django web framework, and Amazon Web Services (AWS) cloud services for hosting and storage are the software.
- The Relational Database Service (RDS) offered by Amazon Web Services for the purpose of storing attendance data.
- An application for mobile devices or the web that may be used to retrieve attendance statistics.

**Requirements from a Functional Perspective:**

- Keeping track of and reporting on attendance in real time
- Automated procedure for managing attendance • Error-free recording of attendance • Attendance data accessible through online or mobile application • Reliable and secure processing and storage of attendance data

**Requirements that aren't related to functionality:**

• An availability rate of at least 99.9 percent for the system

- high system performance for real-time attendance monitoring and reporting; • scalability for handling considerable volumes of attendance data; • security measures for protecting attendance data against unwanted access or change.

**Components of the System and Their Modules**

The hardware component of the proposed systemconsists of the RC522 RFID module (Figure 4.), Arduino Uno R3 board (Figure 5.), and RFID enabledtags or cards (Figure 6.) The hardware component, the software component, and the cloud component make up this architecture for the attendance management system. This architecture is intended to increase the effectiveness and precision of traditional attendance systems via the automation of the process of managing attendance as well as by improving efficiency. All of these individual components are created in such a way that allows the overall design to conform to the metrics that were initially taken into account. Accuracy, efficiency, reliability, and cost-effectiveness are some of the metrics that are taken into account while evaluating the system.The RC522 RFID module, the Arduino Uno R3 board, and RFID-enabled tags or cards are what make up the hardware component of the system that is being suggested. Reading the RFID-enabled tags or cards worn by users allows the RFID module to collect attendance data. This is done by reading the tags or cards. The data are transmitted from the module to the Arduino Uno board, where they are processed before being sent on to the software component. Users are provided with RFID-enabled tags or cards, and then they record their attendance by simply scanning the tag or card at the terminal that has been allocated for that purpose.

**Components of the Software**

The Python Django web development framework is being utilized throughout the construction of the software component of the proposed system. For the purpose of developing online applications, Django offers a comprehensive set of capabilities. These technologies, which include authentication, data modeling, and data security, are crucial components of the attendance management system. Through the use of the local network, the software component is linked to the Arduino Uno R3 board. This board then establishes communication with the RC522 RFID module in order to obtain the attendance data. The attendance records are kept in a database that is part of the software component. Authorized users of the system are the only ones who can access this database and view the attendance records. A web-based administrative user interface is also provided by the software component. With this interface, authorized users are able to monitor attendance records in real time, produce reports (in formats such as.xlsx and.csv), and manage attendance records.

**Component of the Cloud**

The suggested system makes use of cloud computing technologies provided by AWS for the construction of the cloud component. The hosting of the attendance management system may be accomplished using AWS, which offers a solution that is scalable, dependable, and cost-effective. The cloud component makes use of a number of Amazon Web Services (AWS) products, such as Amazon EC2 for the hosting of the web application, Amazon RDS for the

storage of the database, Amazon S3 for the storage of the generated reports (.xlsx,.csv), and Amazon Elastic Load Balancer (ELB) for scalability. The data on attendance is sent from the software component of the system to the cloud component, where it is processed and stored. The cloud component is responsible for both processing and storing the data. The cloud feature offers a centralized place for storing the attendance data, which can be viewed from any location with an internet connection. This feature also allows for the data to be updated in real time. In addition, the cloud component offers a scalable solution that can meet the expansion of the attendance management system as the number of users and attendance data grows. This growth may be accommodated by the scalability of the solution.

**System Design and Construction Procedures**

An RFID tag reader RC522, an RFID tag, and an Arduino Uno R3 board make up the hardware component of the system, respectively. The user is able to be uniquely identified thanks to the RFID reader, which records the one-of-a-kind identification number stored on the RFID tag. The information gleaned from the RFID reader is processed by the Arduino UNO board and then uploaded to the cloud. The data is initially transmitted to the institution's local network, and then, via the institution's primary router or gateway, the data packet is delivered to the Clod gateway together with the API KEY. The software component is being operated on a number of different AWS cloud infrastructures; by combining all of these, we have created our software component, which is responsible for handling all of the data analysis, creating reports, and providing the administrator user with the ability to control and use it. We are able to provide a demonstration of the entirety of the software component's architecture. After the one-of-a-kind identifier has been delivered to the AWS access point using the API KEY, we go on to the further steps, which include reviewing the data of legitimate tag holders and authenticating the data if it was not determined to be in the correct format. In the event that the attendance is not made to the AWS RDS, our authentication procedure will either send a 400 message to the hardware or it will make the attendance there. In addition, the program has a cloud storage component for the purpose of keeping the generated reports and, if necessary, additional system data for either short-term or long-term usage, depending on the circumstances.

The Python Django application, the Amazon Elastic Compute Cloud (EC2), and the Amazon API Gateway make up the three primary components that make up the software component. Diagram of the movement of cloud components. The data that is sent from the Arduino Uno board is received by the AWS EC2 and then stored in the database that is associated with the AWS RDS. The requests and answers that occur between the AWS EC2 and the Python Django application are managed through the usage of the AWS API Gateway. Both the presentation of the attendance data and the generation of reports are the responsibilities of the Python Django application. In the end, it uses Amazon Simple Storage Service (S3) to store all of the previously generated reports and data. Setting up the cloud services provided by AWS, configuring the RFID module to work with the Arduino Uno board, and developing the Python Django application are the three steps involved in the implementation of the system. AWS EC2, AWS API Gateway, AWS Elastic Load Balancer, AWS S3, and AWS RDS are the AWS services that are necessary for the system to function properly. The software on the hardware module is set up to transmit the data to the AWS API Gateway together with the API KEY for the purpose of authentication. The API Gateway subsequently saves the data in the AWS RDS database. The Python Django application is constructed to retrieve the data that is stored in the AWS RDS database and present it on the management user interface in a way that is friendly to users.
One micro service on an EC2 server is responsible for providing all of the static files located in the 0.0.0.0:80/static directory, and the second micro service is responsible for delivering the dynamic Django web application on the same port. Both of these micro services are redirected through the settings of the Apache Web Server. We also have the option of using NGINX, which is another widely used web server.Figure 7. Proposes the attendance management system that utilizes IoT, RFID technology, and AWS cloud services to provide an accurate and efficient attendance management solution.

The Internet of Things (IoT), radio frequency identification (RFID), and Amazon Web Services (AWS) cloud services are utilized by the attendance management system that provides an accurate and effective solution for managing attendance. Seeing as how we have previously gone over the fundamentals of each module and their design; these flow diagrams will not be repeated here. Describes the overall structure of the architecture in its entirety. The

system's hardware component takes the attendance data, and the software component saves and Architecture processes the data on the AWS cloud. Setting up the AWS services, configuring the hardware, and developing the software application are all necessary steps in the process of putting the system into operation

## RESULTS AND DISCUSSION

**Key Findings and Contributions**

A whole new architecture for an attendance management system is offered; it makes use of Internet of Things, Amazon Web Services, and RFID technology, and it is controlled by an Arduino Uno board. Traditional methods of managing attendance may be improved with the help of this system, which offers a solution that is both efficient and cost-effective. The suggested architecture is comprised of hardware and software as its two basic building blocks. The hardware component consists of an RFID module that is linked to an Arduino UNO R3 board. This board is responsible for recording attendance information. The software component was developed with Python Django and is hosted on the AWS cloud. It is responsible for storing, processing, and producing the attendance data in the file format of either.xlsx or.csv. It will be much simpler for administrators to keep track of attendance and create reports using the system that has been proposed since it would allow real-time tracking and reporting of attendance that can be accessed via a web application or a mobile application.

The system was put into action and put through its paces in a real-world setting employing RFID-enabled tags or cards to take attendance. The findings demonstrate that the system is superior to standard attendance management systems in terms of both its accuracy and its efficiency. The solution that has been developed has the potential to be used in a variety of businesses, which would result in enhanced procedures for attendance management and higher levels of productivity. The issues that are experienced by standard methods of attendance management are addressed and a novel solution is proposed in this research study, making the work's contributions particularly noteworthy. The approach that has been suggested is not only more accurate and effective, but it is also less expensive and simpler to put into action. This research reveals unexplored avenues for the application of internet of things (IoT) and cloud computing technologies in attendance management systems and lays forth a strategy for further investigation into this subject area.

**Applications**

The study findings reported in this paper have significant implications and applications for organizations in many sectors, particularly those that require efficient attendance management, such as the education and business industries. These implications and applications have enormous value for these companies. The research proposes an attendance management system that is novel, reliable, and efficient. This system has the potential to improve productivity and decrease expenses. The capacity of the system to monitor and report attendance in real-time enables companies to effectively monitor attendance patterns, hence simplifying the discovery of trends that might potentially enhance overall attendance and productivity. Moreover, the use of Internet of Things (IoT), Amazon Web Services (AWS), and Radio Frequency Identification (RFID) technologies in the suggested framework demonstrates the significant impact these developing technologies may have on enhancing conventional procedures, particularly in the realm of attendance management. Organizations can get advantages by utilizing this technology to automate more laborious and error-prone procedures, resulting in increased operational efficiency and accuracy. The suggested attendance management system exhibits a high degree of scalability and adaptability, making it suitable for implementation in a wide range of organizational contexts, including both small firms and large-scale organizations. The system's availability via online and mobile applications enables enterprises to effectively oversee attendance from any geographical location, making it well-suited for meeting the demands of remote work settings.

The suggested attendance management system possesses a wide range of implications and applications, providing enterprises with a cost-effective, accurate, and effective alternative for managing attendance. The proposed solution possesses the capacity to greatly augment overall productivity and profitability inside companies, hence reinforcing its strategic significance within the current landscape of organizational operations.

**Future Prospects**

The proposed attendance management system, which makes use of IoT, AWS, and the RFID module, offers encouraging prospects for further development and improvements. An approach that can be taken is to enhance the capabilities of the system through the integration of sophisticated features such as biometric authentication or facial recognition. The integration of this feature would enhance the monitoring of attendance, thereby guaranteeing increased levels of security and precision. An additional aspect that could be enhanced pertains to the incorporation of machine learning algorithms. The implementation of machine learning techniques could facilitate the predictive analysis of attendance patterns and the timely detection of potential absenteeism concerns, thereby augmenting the predictive capabilities of the system. Furthermore, in order to enhance scalability, forthcoming endeavors may involve the deployment of a distributed architecture. Implementing this enhancement would allow the system to effectively accommodate a greater number of users and process a more comprehensive dataset. Furthermore, the integration of the system with other enterprise systems, including payroll and HR administration, can serve to expand its scope. The establishment of such integration would facilitate the optimization of organizational processes through the development of a more all-encompassing ecosystem that enables smooth data transfer and integration. The improvement proposals have the potential to greatly enhance the precision, effectiveness, and expandability of the attendance management system. In conclusion, this advancement will augment the system's practicality in various organizational contexts, further harmonizing it with the ever-changing requirements of the modern business environment.

## CONCLUSION

An innovative design has been developed for an attendance management system that makes use of the Internet of Things (IoT), Amazon Web Services (AWS), an RFID module, Python Django, and Arduino Uno. This architecture provides a leading solution for attendance monitoring that encompasses increased accuracy, efficiency, and cost-effectiveness. The possibility of its adoption across a wide variety of organizational landscapes holds the promise of improved attendance management procedures, which will ultimately result in increased levels of productivity. The capability of the system to give real-time attendance tracking and reporting makes the process of monitoring and managing attendance in businesses a considerable amount simpler. In addition, the system that has been offered stands out due to its scalability, flexibility, and user-friendliness of its interface. These characteristics position it as a versatile solution that is suited for a variety of organizational settings.

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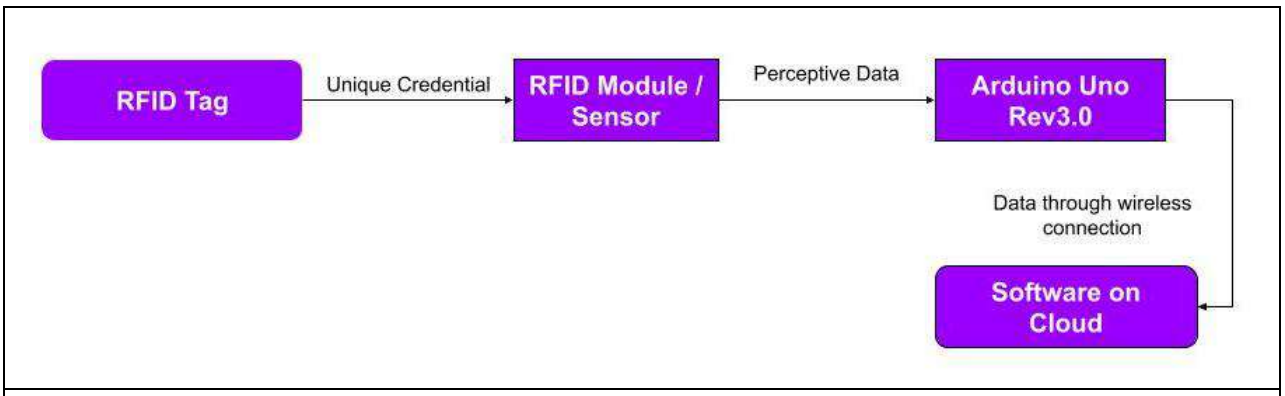


**Fig 1. Hardware components flow diagram**

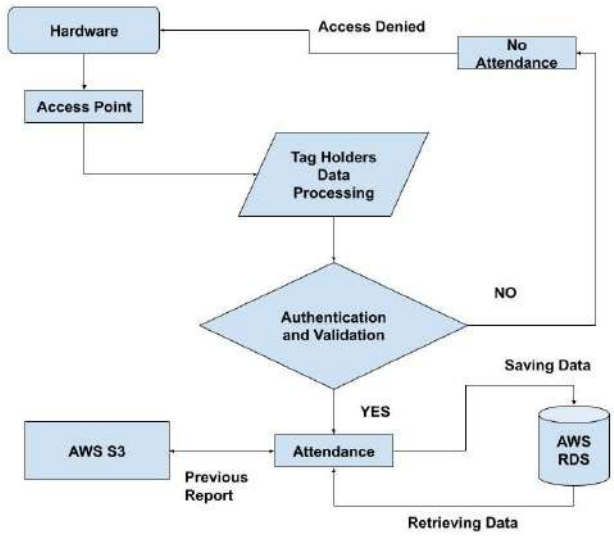


**Fig 2. Software components flow diagram**

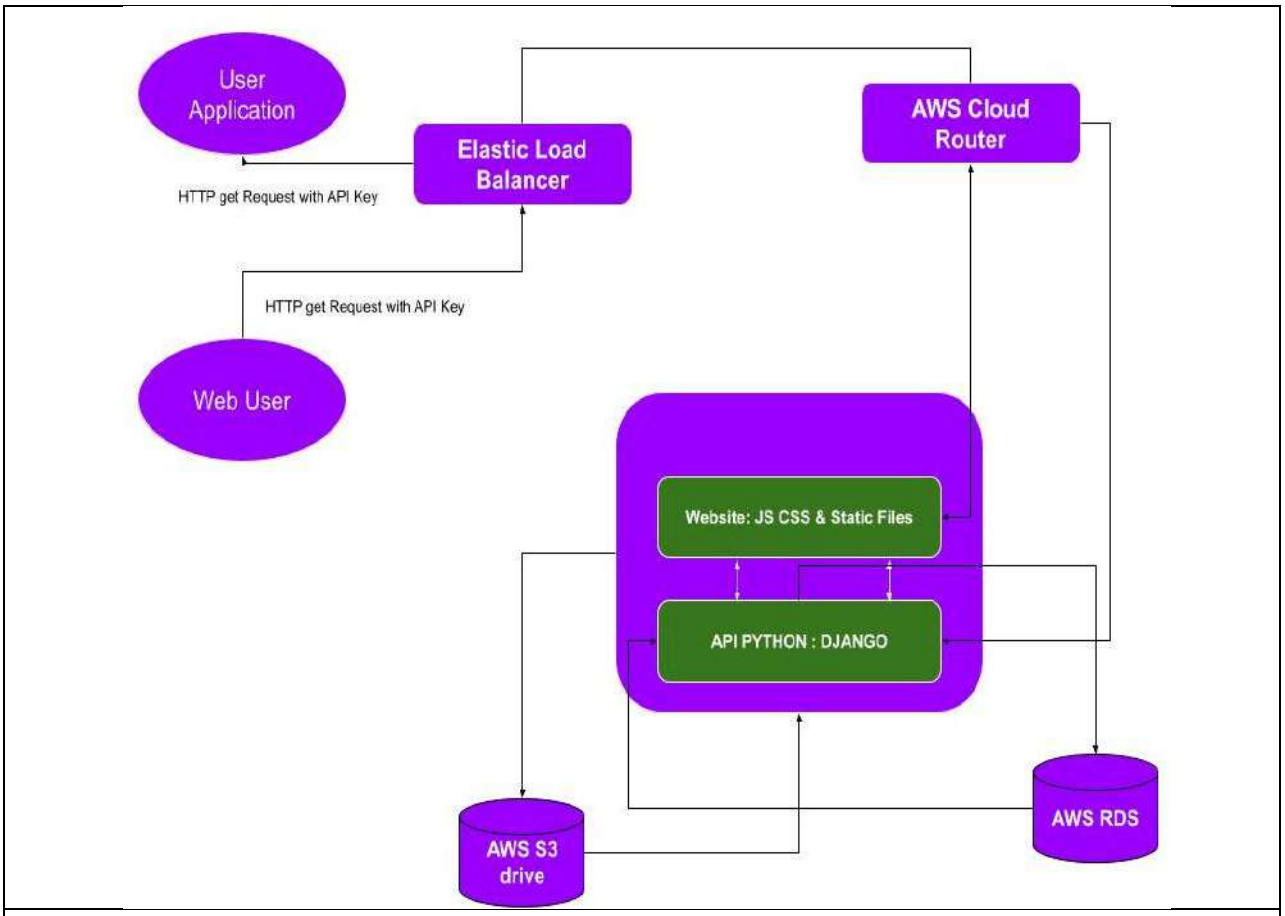


**Fig 3.Cloud components flow diagram**

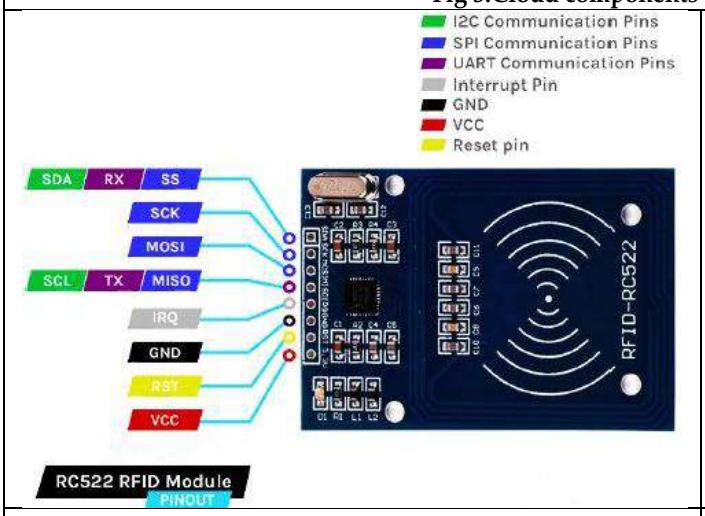


**Fig 04. RC522 RFID Module**

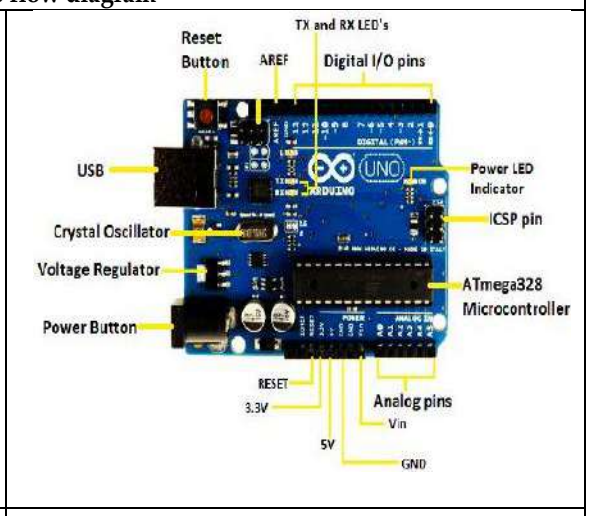


**Fig 5. Arduino UNO R3**

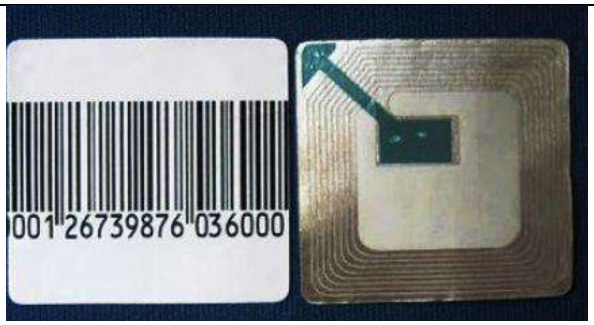


**Fig** 6. RFID Tag or Card

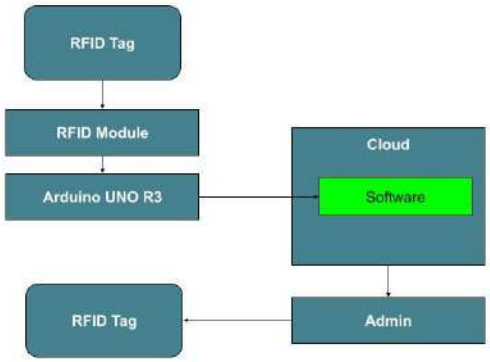


**Fig.7. Proposed Architecture**

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**RESEARCH ARTICLE**

# Effect of Various Organic Fertilizers in Growth and Yield of Turnip (*Brassica rapa* L.) Under Low Hills of Uttarakhand

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## ABSTRACT

During 2022-23, a field experiment was planned and conducted at the Horticulture Research Block, School of Agriculture Sciences, SGRRU, Dehradun, Uttarakhand, to investigate the "Effect of various organic fertilizers in growth and yield of Turnip (*Brassica rapa* L.) under low hills of Uttarakhand." The experiment was laid out in a randomized block design with three replications and ten treatments. The treatments comprised the following levels of different organic manures with different concentrations: T1: control (soil@100%), T2: (FYM@25tons/ha), T3: (Vermicompost @6tons/ha), T4: (Jeevamrit@100%), T5: (Biostimulant@3%), T6: FYM + Vermicompost (25 tons/ha + 6 tons/ha), T7: Vermicompost + Jeevamrit (6 tons/ha + 100%), T8: FYM + Biostimulant (25 tons/ha + 3%), T9: FYM + Vermicompost + Jeevamrit + Biostimulant (12.5 tons/ha + 3 tons/ha + 50% + 1.5%) and T10: FYM + Vermicompost + Jeevamrit + Biostimulant (25 tons/ha + 6 tons/ha + 100% + 3%).The sowing of Turnip "Purple Top White Globe" was done on 18/11/2022, and the final harvest was conducted on 11/02/2023. Observations on various attributes such as growth, yield, quality, and economics were recorded.The results revealed that treatment T6 (FYM + Vermicompost (25 tons/ha + 6 tons/ha)) was found to be the most effective in terms of vegetative characters such as plant height (45.93 cm), number of leaves (17), leaf length (26.82 cm), leaf width (13.02 cm), fresh weight of leaves (64.39 g), and dry weight of leaves (16.12 g). Yield attributes were also the highest for T6, including root diameter (11.53 cm), root length (9.25 cm), root weight (109.83 g), yield (17.46 kg/plot) and total yield (178.2 q/ha).

**Keywords:** Turnip, randomized, vermicompost, biostimulant, Jeevamrit

## INTRODUCTION

Turnip, scientifically known as *Brassica rapa* L., is a globally important root crop and a close relative of Arabidopsis. It belongs to the family Brassicaceae and Cruciferae, with a chromosome number of 2n=20. Turnip has been cultivated for about 2000 years across a wide range of climates, from Western Europe to China and Norway to the African desert (Nawaz *et.al*., 2019). Various common vegetables are included in the genus Brassica, including *Brassica rapa* L., also known as shaljam. Since prehistoric times, turnip has been used for human consumption and is one of the oldest cultivated vegetables, particularly famous in Europe and thriving in temperate climates (Mourato*et.al*., 2015). Vegetables play a major role in Indian agriculture by providing nutritional and economic security and yielding higher returns per unit area and time. India, largely a vegetarian society, depends heavily on vegetables for the bulk of its nutritional requirements. Since independence, India has emerged as the second-largest producer of vegetables in the world, after China. Vegetable production has increased from less than 20 million tons before independence to 212.53 million tons, and productivity has increased to 17.3 million tons/ha in 2022-23. The area under vegetable cultivation has grown from 5.5 million ha in 1991-92 to 11.477 million ha in 2022-23. According to the National Horticulture Database 2011, India is the largest producer of ginger and okra and ranks second in the production of potatoes (10%), onions, cauliflower, and cabbage, earning the moniker of the fruit and vegetable basket of the world. Over the last five years, an overall growth rate of 2.08% in area, 1.64% in productivity, and 3.72% in total production has been achieved. The demand for vegetables in India is projected to reach 350 million tons by 2030 (Singh, 2019).Turnip is a vital root vegetable and forage crop, indigenous to Asia, Europe, Russia, and the Near East, and is now widely cultivated globally as both a vegetable and oil source. In Pakistan, turnip was cultivated on 9609 hectares of land with a production of 167,065 tons in 2020-2021, making it an important local vegetable. Turnip has been reported to have various medicinal benefits, including therapeutic properties against kidney and liver diseases and other ailments. It also possesses antimicrobial, anti-inflammatory, antitumor, cardio protective, anti-diabetic, analgesic, and nephroprotective properties, and aids against metabolic syndrome and obesity. Furthermore, it improves growth in response to heavy fertilization, which can cause non-point pollution (Raza *et.al*., 2023).Turnip is commonly grown in temperate climates for its white, fleshy taproot and is one of the oldest cultivated vegetables. It is an excellent source of bioactive compounds, potentially delivering health benefits when consumed (Jadid*et.al*., 2021). Brassica, the most important genus in the Brassicaceae family, consists of about 350 genera and nearly 3,500 species. Young turnip roots are commonly consumed raw in salads, and turnip leaves, characterized by a bitter taste due to trace amounts of phenolic compounds, differ from other Brassica vegetables in their trivial antioxidant capacity (Dejanovic*et.al.,* 2021).In the UK, turnip, a traditional root vegetable, is less frequently consumed compared to other Brassica vegetables. In 1992, turnip production accounted for 5400 hectares, which dropped to less than 2700 hectares by 2017 (Nor *et.al*., 2020). According to the Food and Agriculture Organization (FAO) of the United Nations, 92 million tons of Brassica plants are grown in 150 countries, occupying 5.4 million hectares of land, with 70% of this area in Asia and China (Chihoub*et.al.,* 2019).The decision to conduct the present experiment with organic fertilizers such as Jeevamrit and biostimulants for turnip cultivation in the low hills of Uttarakhand is driven by the scarcity of research in this area. Recognizing the lack of studies on the efficacy of these specific fertilizers on turnip crops in this region, this experiment seeks to address this knowledge gap. By investigating the impact of Jeevamrit and biostimulants on turnip cultivation in the unique environmental conditions of Uttarakhand's low hills, the study aims to provide valuable insights that can benefit local farmers and contribute to sustainable agricultural practices in the region.

## MATERIALS AND METHODS

The present research was conducted at the Horticulture Research Block, Department of Horticulture, School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, during the rabi season of 2022-23. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Ten treatments were tested: $T_1$

(control), $T_2$ (Farm Yard manure @22t/ha), $T_3$ (Vermicompost @ 5t/ha), $T_4$ (Jeevamrit @100%), $T_5$ (Biostimulant @3%), $T_6$ (FYM @22t/ha + Vermicompost @5t/ha), $T_7$ (Vermicompost @5t/ha + Jeevamrit @100%), $T_8$ (FYM @22t/ha + Biostimulant @3%), $T_9$ (FYM @11 t/ha + Vermicompost @2.5 t/ha + Jeevamrit @50% + Biostimulant @1.5%) and $T_{10}$ (FYM @22t/ha + Vermicompost @5t/ha +Jeevamrit @100% + Biostimulant @3%). The soil of the research field was sandy loam with a pH of 7.12, containing available nitrogen (220.04%), available phosphorus (9.1 kg/ha), and available potassium (18.1 kg/ha). The turnip cultivar "Purple Top White Globe" was selected for this study. Organic fertilizers (Vermicompost, FYM, Jeevamrit, and Biostimulant) were incorporated into the experimental field as per the treatment specifications during the final field preparation. Seeds were sown on 18/11/2022, and all necessary cultural practices were carried out at regular intervals throughout the research period.During the experiment, five plants were randomly selected from each replication to record various observations on growth and yield characteristics at 30 days, 60 days after sowing, and at final harvest. The data obtained were statistically analyzed using standard statistical methods as suggested by Gomez and Gomez (1996).

## RESULT AND DISCUSSION

**Plant height**

The data recorded on height of plant at different harvesting are presented in the Table 2 and Fig. 1.At 30 DAS, the maximum plants height (15.78 cm) was recorded in T10. The treatment T9 (14.41 cm) and T8 (14.49 cm) was at par with each other. However, significant differences were observed with treatment T1 (11.21 cm), T6 (13.16 cm) and T8 (14.49 cm). The minimum plant height was recorded in T3 (9.59 cm).At 60 DAS the maximum plants height (34.99 cm) was recorded in T5, which at par T6 (34.14 cm), T7 (34.02 cm) and T10 (34.13 cm). However, significant differences were observed with treatment T1 (29.56 cm), T2 (28.3 cm), T3 (31.11 cm), T4 (32.3 cm) and T10 (34.13 cm). The minimum plant height was recorded in T2 (28.3 cm).At final harvest the maximum plants height was recorded in T4 (45.93 cm) with FYM @ 5 tones/ha + V.C @ 2.5 tones/ha which was at par with T6 (45.68 cm) and T10 (45.56 cm). However, significant difference was observed with treatment T3 (42.88 cm), T9 (43.66 cm), T8 (35.92 cm) and T2 (37.79 cm).While, the minimum plant height was recorded in T1 (35.74 cm). The probable reasons for enhanced a greater plant height may be due to promotive effect of macro and micro nutrients on vegetative growth ultimately led to more photosynthetic activities. The findings are in agreement with Sapkota*et.al*., 2021; Singh *et.al.*,2020 and Yadav *et.al*., 2020.

**Number of leaves per plant**

The data recorded on height of plant at different harvesting are presented in the Table 2 and Fig. 2. At 30 DAS the number of leaves per plant ranged from 5 to 6. On the basis of mean the maximum number of leaves per plant was counted in T1, T2 and T10 (6) which at par with each other. However, significant differences were observed in T1 (6) and T3 (5). The minimum number per plant was recorded in the treatment T7 and T8 (5).In the case of 60 DAS, the mean value for number of leaves per plant was found maximum in T9 (12). The treatments T1 (11), T4 (11), T6 (11), T7 (11) and T10 (11) was at par with each other. However, significant differences were found in T1, T3 and T9. The minimum number of leaves per plant was recorded in the treatment T7 or T8 (5).At final harvest after sowing significant differences and on the basis of mean the maximum number of leaves per plant were counted in the treatment T8 (17) which were at par with T2 (17). However, significant differences were found with rest of the treatment T1 (15), T4 (14), T5 (15) and T7 (13). The minimum number of leaves per plant was recorded in the treatment T3 (12).The probable reasons for enhanced a greater number of leaves may be due to promotive effect of macro and micro nutrients on vegetative growth ultimately led to more photosynthetic activities. Similar findings are alsoreported by Kiran *et.al*., 2017 and Rawat*et.al*., 2021.

**Leaf length**

The observation of leaf length was recorded at 30, 60 DAS and at final harvest the results show significant differences between the treatments.At 30 DAS, the maximum value of leaf length was recorded in the treatment T4 (8.92 cm) which were at par with T5 (8.57 cm), T6 (8.34 cm), T9 (8.00 cm) and T10 (8.52 cm). The significant differences were

observed with the treatments T1, T2, T3, T4, T5 and T6. The minimum value (7.1 cm) of leaf length was recorded under the treatment T3.At 60 DAS, the maximum number of leaf length was recorded in treatment T8(23.89 cm). The treatments T5, T6, T7, T8 and T9 were at par with each other. The significant differences were observed with the treatment T1 (18.16 cm), T3 (20.78 cm), T4 (19.75 cm), T8 (21.75 cm) and T10 (22.45 cm). The minimum leaf length (18.16 cm) was recorded under the treatment T1.At final harvest, the leaf length was maximum in T10 (33.50 cm) which was at par with T4 (26.44 cm). However, significant differences were observed with treatment T1 (20.81cm), T3 (22.11cm),) T6 (25.68 cm)and T8 (22.46 cm). The minimum leaf length was recorded in the treatment T2 (19.47 cm).The application of FYM @ 5 tones/ha + Vermicompost @ 2.5 tones/ha + V.W @ 25 increases the leaf length of turnip may be due to application of major and minor nutrients, through organic manures in various levels, increased the photosynthetic activity, chlorophyll formation, nitrogen metabolism and auxin contents in the plants which ultimately increases the plant height. The findings are also in conformity with the findings of Joshi *et.al*.,2015.

**Leaf width**

The leaf width of the plant at different stages of harvesting is presented in Table 2.and Fig. 4.The leaf width on 30 days after sowing differs significantly and was ranging from 3.7 to 4.85 cm. The maximum leaf width was recorded in T6 (4.85 cm) which was statistically at par with T5 (4.66 cm) and T4 (4.53 cm). However, significant differences were observed with the treatments T1 (3.9 cm), T2 (4.01 cm), T3 (3.71 cm). The minimum leaf width was recorded in the treatment T1 (3.9 cm).In 60 DAS, the maximum leaf width was recorded in T4 (10.81 cm) which was at par with treatment T6 (10.02 cm). However, significant differences were observed with treatment T1 (8.50 cm), T4 (10.81 cm), T5 (9.69 cm) and T9 (8.7 cm).At final harvest, **t**he maximum leaf width (13.02 cm) was recorded in the concentration of organic manures treatment T4 (Vermicompost @ 5 tones/ha) which is found to be on per with treatment T1. The minimum was recorded in this might be due to the continuous nutrient availability by the use of organics. This is found to be accordance with findings of Dlamini*et.al*.,2020.

**Root length**

The maximum root length was recorded in treatment T4 (9.25 cm) with the application of Vermiwash @ 50 %. The minimum root length was recorded in T3. The significantly highest root length might be due to beneficial effect of organic nutrient sources. The organic nutrient sources, particularly vermicompost improves the soil structure and soil quality which might have resulted in length of root. The findings are in similar with the results of Joshi *et.al*.,2015; Aisha *et.al*., 2014 and Pamula*et.al*., 2020.

**Root weight**

The maximum root weight of turnip (135.74 g) was recorded in T10 with FYM @ 5 tones/ha +Vermicompost @ 2.5 tones/ha + Vermiwash @ 25% significantly superior to all other treatments. The minimum root weight was recorded in T7. The results show significant differences between the treatments. The increase in root weight of leaves may be due to the excellence of high level of organic manures was producing good growth of turnip plants which show higher root weight. The findings are in similar with Getaneh*et.al*.,2019 and Joshi *et.al*.,2015.

**Root diameter**

The maximum root diameter was recorded in T7 (8.35 cm) with FYM @ 5 tones/ha + V.C @ 5 tones/ha which was at per with T4 significantly superior to all treatments. The minimum root diameter was recorded in T3. The decrease in bulk density and increase in porosity and water holding capacity of the soil due to organic nutrient source might have contributed in increasing root diameter of plants. The results are similar with Pamula*et.al*.,2020 and Kon*et.al*., 2016.

**Fresh weight of leaves**

The maximum total fresh weight of leaves (66.44 g) was recorded in T4 with Vermiwash @ 50% which was significantly superior to all other treatments. The minimum total fresh weight of leaves was recorded in T8. The results show significant differences between the treatments. The increase in total fresh weight of leaves may be due to

the excellence of high level of organic manures was producing good growth of turnip plants which show higher total fresh weight of leaves. The findings are in accordance with Basheer *et.al*.,2013.

**Dry weight of leaves**

The maximum total dry weight of leaves (16.12 g) was recorded in T4 with vermiwash @ 50 % which was significantly superior to all other treatments. The minimum total dry weight of leaves was recorded in T4. The results show significant differences between the treatments. The increase in total dry weight of leaves may be due to the excellence of high level of organic manures was producing good growth of turnip plants which show higher total dry weight of leaves. Similar results were also reported by Joshi *et.al*.,2015 and Kaur *et.al*., 2019.

**Root yield (kg/plot)**

The maximum root yield of turnip (17.46 kg) was recorded in T10 FYM @ 5 tones/ha +Vermicompost @ 2.5 tones/ha + Vermiwash @ 25% + Jeevamrit @ 50 %which was significantly superior to all other treatments. The minimum root yield was recorded in T5. The results show significant differences between the treatments. The increase in root yield may be due to the excellence of high level of organic manures was producing good growth of turnip plants which show higher root yield. The findings are in agreement with Jadhav*et.al*.,2015; Getaneh*et.al*., 2019 and Yogeshappa*et.al*., 2017.

**Root yield (t/ha)**

The maximum root yield of turnip (17.15 t/ha) was recorded in T3 with FYM @ 10 tones/ha significantly superior to all other treatments. The minimum root yield was recorded in T5. The results show significant differences between the treatments. The increase in root yield may be due to the excellence of high level of organic manures was producing good growth of turnip plants which show higher total root yield. The findings are parallel with Getaneh*et.al*.,2019 and Ghimire*et.al*., 2020.

## CONCLUSION

Based on the current investigation on the "Effect of Various Organic Fertilizers on the Growth and Yield of Turnip (*Brassica rapa* L.) Under Low Hills of Uttarakhand," it can be concluded that the treatment T6 (Farmyard Manure@ 25 tons/ha + Vermicompost @6 tons/ha) resulted in the best performance. Specifically, the following outcomes were observed with this treatment: Growth attributes viz., maximum plant height (45.93cm), number of leaves (17), leaf length (26.82cm), leaf width (13.02cm), fresh weight of leaves (64.39g ) and dry weight of leaves (16.12 g) and yield attributes viz., root diameter (11.53 cm), root length (9.25 cm), root weight (109.83 g), yield (17.46 kg/plot) and total yield (178.2 q/ha).The study indicates that the combined use of FYM and Vermicompost, supplemented with Jeevamrit, significantly enhanced soil fertility, which in turn led to improved yield and quality of turnip crops. Therefore, under the current agro-climatic conditions of the low hills of Uttarakhand, it is recommended to use this combination of organic fertilizers to achieve sustainably higher yields and quality in turnip cultivation.

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**Table:1 Treatment details**

| No. of Treatment | Combinations | Concentration |
|---|---|---|
| $T_1$ | Control | - |
| $T_2$ | FYM | 25tones/ha |
| $T_3$ | Vermicompost | 6tones/ha |
| $T_4$ | Jeevamrit | 100% |
| $T_5$ | Biostimulant | 3% |
| $T_6$ | FYM + Vermicompost | 25tones/ha + 6 tones/ha |
| $T_7$ | Vermicompost + Jeevamrit | 6 tones/ha + 100% |
| $T_8$ | FYM + Biostimulant | 25tones/ha + 3% |
| $T_9$ | FYM+Vermicompost+ Jeevamrit + Biostimulant | 12.5tones/ha+3tones/ha +50%+1.5% |
| $T_{10}$ | FYM+Vermicompost+jeevamrit+ Biostimulant | 25tones/ha+ 6 tones\ha+100 %+ 3% |

**Table 2: Effect of organic fertilizers on plant height (cm), number of leaves per plant, leaf length (cm) and leaf width cm) of Turnip at different harvest intervals**

| Treatment | Plant height (cm) | | | Number of leaves per plant | | | Leaf length (cm) | | | Leaf width (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 DAS | 60 DAS | At Final harvest | 30 DAS | 60 DAS | At Final harvest | 30 DAS | 60 DAS | At Final Harvest | 30 DAS | 60 DAS | At Final Harvest |
| $T_1$ | 11.21 | 29.56 | 35.74 | 6 | 11 | 15 | 7.48 | 18.16 | 21.06 | 3.9 | 8.50 | 12.3 |
| $T_2$ | 10.67 | 28.3 | 37.79 | 6 | 10 | 16 | 7.24 | 18.82 | 19.47 | 4.01 | 8.95 | 11.17 |
| $T_3$ | 9.56 | 31.11 | 42.88 | 5 | 10 | 12 | 7.1 | 20.78 | 22.11 | 3.71 | 8.90 | 10.54 |
| $T_4$ | 11.86 | 32.3 | 45.93 | 6 | 11 | 14 | 8.92 | 19.75 | 23.64 | 4.53 | 10.81 | 13.20 |
| $T_5$ | 11.61 | 34.99 | 44.66 | 6 | 10 | 15 | 8.57 | 16.21 | 25.40 | 4.66 | 9.69 | 11.44 |
| $T_6$ | 13.16 | 34.14 | 45.68 | 6 | 11 | 14 | 8.34 | 21.32 | 25.90 | 4.85 | 10.02 | 10.7 |
| $T_7$ | 13.51 | 34.02 | 41.96 | 5 | 11 | 13 | 7.81 | 21.26 | 30.30 | 4.37 | 9.43 | 11.2 |
| $T_8$ | 14.49 | 31.61 | 35.92 | 5 | 10 | 17 | 7.73 | 23.89 | 29.50 | 4.3 | 9.44 | 11.68 |
| $T_9$ | 14.41 | 32.21 | 42.66 | 5 | 12 | 13 | 8.00 | 21.53 | 21.53 | 3.97 | 7.71 | 9.62 |
| $T_{10}$ | 15.78 | 34.13 | 45.56 | 6 | 11 | 15 | 8.52 | 22.45 | 33.50 | 4.11 | 8.03 | 10.62 |
| C.D(0.05%) | 0.714 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 1.135 | N/A | N/A |
| SE(m) ± | 0.238 | 5.46 | 6.07 | 1.3 | 1.54 | 2.31 | 0.326 | 2.33 | 3.78 | 0.379 | 0.84 | 1.79 |
| SE(d) ± | 0.337 | 7.73 | 8.59 | 3.5 | 2.18 | 3.26 | 0.461 | 3.30 | 2.34 | 0.536 | 1.18 | 2.53 |
| C.V. | 1.495 | 1.31 | 1.10 | 20.3 | 2.27 | 2.23 | 0.51 | 2.40 | 2.96 | 1.14 | 1.98 | 1.41 |

**Table 3: Effect of organic fertilizers on fresh weight of leaves (g), dry weight of leaves (g), length of root (cm), diameter of root (g), weight of root (g), root yield (kg/plot) and root yield (q/ha) of turnip at final harvest**

| Treatment | Fresh Weight of leaves (g) | Dry weight of leaves (g) | Root Length (cm) | Diameter of Root (cm) | Root weight (g) | Root yield (kg/plot) | Root yield (q/ha) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 33.67 | 15.49 | 9.06 | 6.40 | 66.95 | 13.36 | 162.2 |
| $T_2$ | 34.36 | 15.34 | 8.20 | 6.76 | 77.12 | 15.06 | 152.5 |
| $T_3$ | 38.99 | 14.13 | 6.79 | 7.73 | 109.83 | 15.20 | 171.5 |
| $T_4$ | 56.92 | 16.12 | 7.59 | 8.53 | 97.59 | 14.70 | 148.5 |
| $T_5$ | 43.72 | 13.22 | 8.15 | 8.86 | 76.88 | 13.40 | 138.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $T_6$ | 64.39 | 15.95 | 7.58 | 7.53 | 74.98 | 17.46 | 178.2 |
| $T_7$ | 51.34 | 14.80 | 7.60 | 8.63 | 69.67 | 17.00 | 145.6 |
| $T_8$ | 46.58 | 15.15 | 8.48 | 9.70 | 85.65 | 14.43 | 178.2 |
| $T_9$ | 56.59 | 14.69 | 8.48 | 11.53 | 79.64 | 15.23 | 146.2 |
| $T_{10}$ | 51.10 | 15.95 | 9.25 | 7.23 | 86.19 | 15.16 | 152.2 |
| C.D (0.05%) | 2.95 | N/A | 1.18 | N/A | N/A | 1.97 | 2.11 |
| SE(m) ± | 2.65 | 0.83 | 0.39 | 1.34 | 12.38 | 0.65 | 0.70 |
| SE(d) ± | 0.75 | 1.18 | 0.55 | 1.89 | 17.51 | 0.93 | 0.99 |
| C.V. | 2.63 | 3.63 | 2.42 | 2.01 | 25.01 | 7.54 | 7.86 |

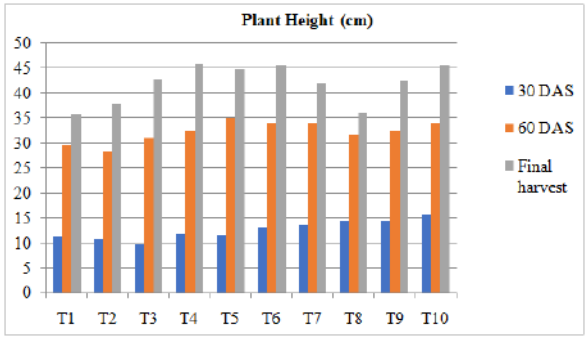


**Fig 1: Plant height (cm) of turnip as influenced by organic manure at various stages of Harvesting**

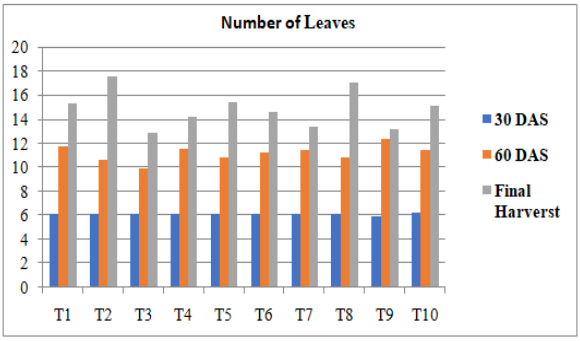


**Fig 2: Number of leaves of turnip as influenced by organic manure at various stages of Harvesting**

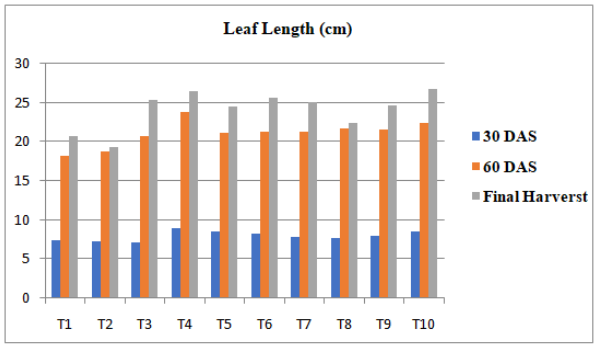


**Fig. 3 : Leaf length (cm) of turnip as influenced by organic manure at various stages of Harvesting**

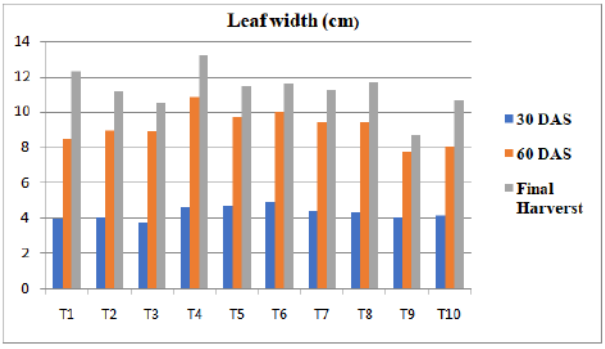


**Fig 4: Leaf width (cm) of turnip as influenced by organic manure at various stages of harvesting**

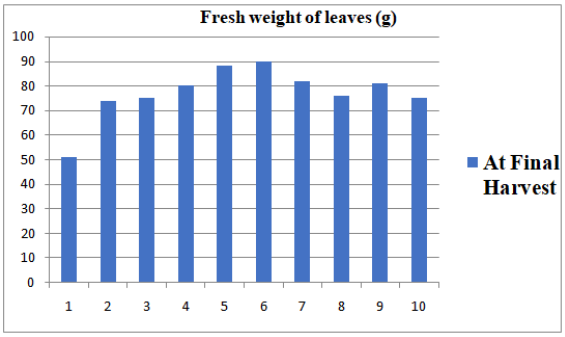


**Fig 5: Fresh weight of leaves (g) of turnip as influenced by organic manure after harvesting**

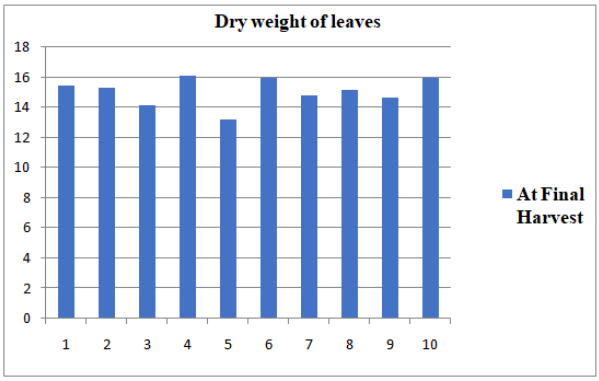


**Fig 6: Dry Weight of leaves (g) of turnip as influenced by organic manure after harvesting**

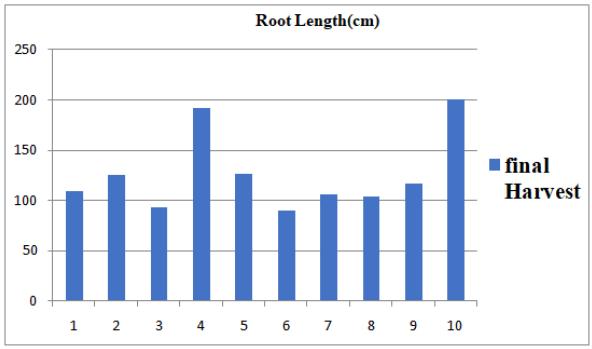


**Fig 7: Root length (cm) of turnip as influenced by organic manure at final stage of harvesting**

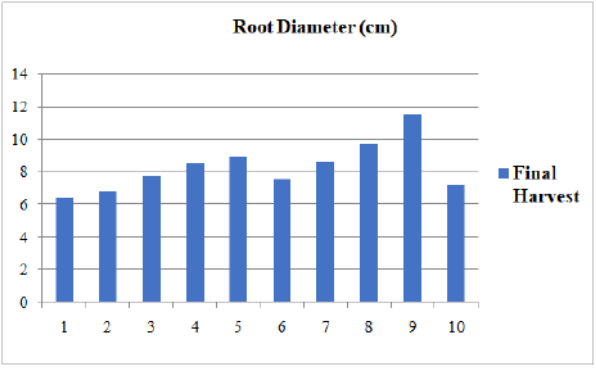


**Fig 8: Root Diameter (cm) of turnip as influenced by organic manure at final stage of harvesting**

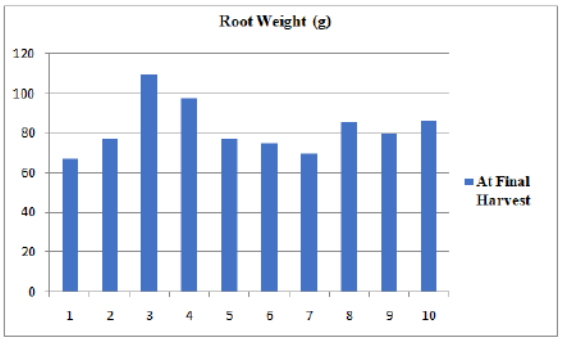


**Fig 9: Root weight (g) of turnip as influenced by organic manure at final stage of harvesting**

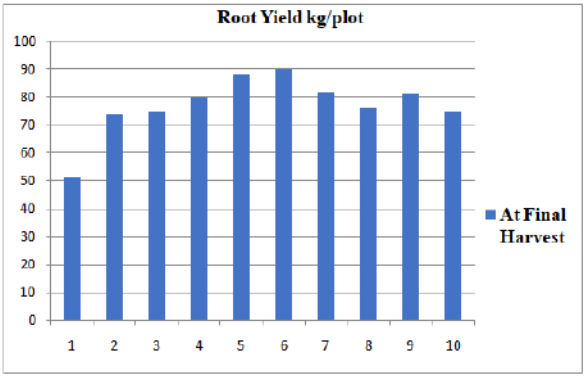


**Fig 10:Effect of organic manures on root yield (kg/Plot) of turnip at final stage of harvesting**

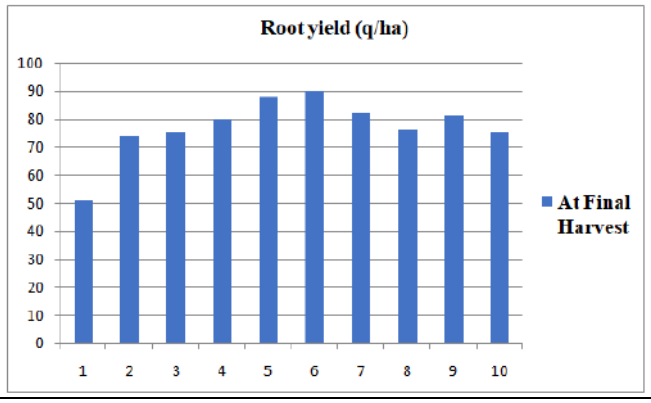


**Fig 11:Effect of organic manures on root yield (q/ha) of turnip at final stage of harvesting**

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**RESEARCH ARTICLE**

# To Study the Relationship between Examination Stress and Changes in Eating Pattern among 18-25 y/o Students of Parul University

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## ABSTRACT

The research study was based on the correlation between exam and stress that may or may not affect eating and sleeping patterns of 18-25 y/o students at Parul university. As these studies started in March 2023 ended in March 2024. Around 303 students (living in hostels or PG) were taken as the target group sample with snowball technique. We have designed an online google form for survey purposes and the questionnaire was broken down into five sections: general information, examination details, food habits, sleeping patterns, and perceived stress (aptitude test). The data collection was conducted in October month from 10th Oct 2023 to 25 oct 2023. A statistical analytical tool (SPSS) has been utilized for data analysis. As students don't worry about the external exam because Parul University administers exams, take the weekly and internal exam that covers half of the curriculum. This observational data relates only to the Parul university students. A total of 303 students answered the questions; upon evaluating the population by gender, it was found that 55.26% of the students were male and 44.7% of the students were female students of Parul University. These students will be appearing for the exam. According to the data we collected, roughly 33% of students had exams, 60% did not, and it is likely that 6% were unaware of the specific examination days. In October, these data were collected. Our research focused mostly on the eating habits of each individual body. Sixty percent of the pupils opt to eat vegetarianism because they are from Gujarat. Fifty percent of pupils experience nutritional modifications, whereas the other fifty percent do not experience any changes in their eating habits. Research indicates that dietary changes may cause sleep patterns to be disturbed, and that studying for college exams is the primary cause of these disturbances. Exams linked to sluggishness, dietary modifications, and PG students' favourite cooking techniques had a positive correlation. This result supports the alternative hypothesis—which shows a strong correlation between exam stress and changes in sleeping and eating patterns—is statistically supported, rejecting the null hypothesis.

**Keywords :** Stress, Emotional behaviour, examination days, correlation data, eating and sleeping pattern.

# INTRODUCTION

In today's world, stress is a prominent factor in every individual's life due to several reason following for the example like studies, relationship, social media influence, financial issue, family problem, office load, etc. WHO defines stress as "State of worry or mental tension caused by a difficult situation". Stress is a natural human response that prompts us to address challenges and threats in us Lives. Everyone experiences stress to some degree. The way we respond to stress, however, makes a big difference to our overall well-being. Stress is nothing but a pressure that build up in the mind to complete any possible task in A particular period of time that changes mental, physical and social life such as a individual diet, social relationship sleeping pattern and psychological factor such as unconscious mind, behaviour changes, feels isolated, don't know the reason of anger, not eating mindfully, overthinking, relationship issue, self-doubt, mental health and trust issue.

The prevalence of stress used to be 37.12 % in 2018 and is continuously increasing after covid-19 pandemic starts in every country that increased stress Up to 43.76 % in 2023. So, it is noticed that there was 6 – 7% increase in stress level. And it is assumed that the level of stress in continuous going to be increased in upcoming years due to competitive exam, job requirement, financial loan, etc. Stress can be classified into short term and long-term duration. So, as short-term duration means it remain for shorter period of time that contain deadline of the project, relationship issue, certain disease includes etc and about long-term duration means that remain more than a year contain disease like cancer, heart & kidney disease, diabetes, hypertension, asthma etc.
Stress is characterized into two types: -

**Acute stress and chronic stress**
Acute stress is comprised of short stress that quickly get reduce by suppress the load ofproblem example Assignment submission, ppt presentation, project deadline, etc. Chronic stress that may comprise of long-term stress that may lead to serious disease example like government exam, yearly university exam, dissertation, etc. This stress may lead to highblood pressure, diabetes, heart disease and obesity. This is due to continuous the presence of stressful life is one of the major changes comes in people lifestyle that are related to eating behaviour.

Most of the times stress build up because of heavy work load, arrival date of assignment submission and exam stress, bank load, home stress, office load, etc. Eating is a major component of lifethat is required in daily routine.Regular eating behaviour is playing a important in nourishing the whole body from maintain the body weight to give proper nutrient to each organ and make used of energy for the whole day. So, if we look around individual in stress are often suffered from lifestyle changes .in which major factor eating behaviour changes too. Eating behaviour / eating disorder are nothing but a psychological disorder in which a person may eat more or eat very less sometimes these maybe caused by extreme stress condition in any situation .Dietary pattern changes when there is excessive amount of stress occur that may lead to low glucose level in the body. Students' lifestyle and dietary pattern is completely disturb especially for those who leave alone without their family member. From making their own work food to complete college is really a tiring job to do so.

Student's stress may lead to eat excess amount of kcal in high quantity and less of nutritive food consumption. That may be a reason of obesity and a imbalance dietary portion size or some may not eat that much in quantity which then lead malnutrition pattern of the body that have condition leads on body example: - Fatigue, numbness in the body and low blood pressure. Research has explored how stress impact eating behaviours in various age groups, including children and adolescents. (Hill et al 2018) (1). Different people are affected by exam stress in different ways. While some people experience intense tension, worry, and uneasiness, others stay calm. Academic pressure is associated with stress, which can cause substantial physical repercussions such as weariness, poor appetite, sleeplessness, and insomnia. Exam pressure and stress are closely related. Depending on the individual, stress can either increase or decrease appetite. It disturbs regular eating patterns and contributes to a number of health problems by having direct biological consequences and indirect behavioural changes that have a detrimental

influence on health. Some physiological factor affects such as fatigue, high hunger, less sleep, sugar craving, food deprivation, illness, drug withdrawal state, anxiety, and also leads to changes in eating behaviours. Exam pressure can significantly impact eating behaviour, which plays a crucial role in maintaining a balanced diet. The correlation between exam stress and eating behavior is essential, especially considering the rising rates of obesity and malnutrition. Understanding this relationship can lead to the development of effective stress reduction methods and awareness of the consequences of stress on dietary patterns. By studying these aspects, we can better comprehend how stress affects our bodies and find ways to modify our behaviors to mitigate its negative effects.

Now a day's there is a huge impact of stress and eating pattern that get visualised into day-to-day life. Not only mental but also physical health burden increase in the communities. This can be led to health issue like anxiety, depression, cardiovascular problem and nutrition deficiencies.(10). As stress has an impact on the body because of the social and relationship problem with friends, family, office colleagues and social media gathering that turn out to be a reason of misleading issue, fights, etc. The influence of social media and unrealistic beauty standards has indeed heightened concerns about body image and health among young people. This pressure can contribute to stress and affect their eating patterns, highlighting the importance of promoting healthy body image and stress management strategies.

## MATERIALS AND METHODS

**Study Design**

In October 2023, we carried out a cross-sectional analytical study on Undergraduate and Postgraduate Students of Parul university, Vadodara, India.

**Study setting & instrument**

The purpose of the study is to demonstrate a relationship between food pattern modifications and exam stress. Utilizing a Google Form, data is gathered using the purposive technique and the snow bowl method. The descriptive study and thorough explanations of the relationship between exam stress and the changes in eating patterns served as the foundation for the survey design. The research was conducted on the localities and hostilities of Parul University students in Vadodara, Gujarat. The first two weeks of October were spent conducting the study. About 304 responses to the survey have been gathered. The minimal age start for students was set at 18 to 25 years old for the purpose of the survey study. Analysis tool used for research paper is SPSS (Statistical package for social science) for data analysis purpose. Two method are used for analysis purpose Chi – square and T- test.

**Participants**

**Inclusion**

1)The respondent know about the concept of stress is a major factor that may their affect eating pattern.
2)The respondent is the student of Parul university and they are living in Vadodara, Gujarat.
3)The respondent is ready to give their consent regarding research topic and proper response to the question.

**Data analysis**

Data entry was done in Microsoft Excel and analysed using IBM SPSS Statistics. We summarized scores as mean with standard deviation (SD) and percentages (categorized). Age and years of experience were correlated with scores using Pearson correlation. wo method are used for analysis purpose **Chi – square** and **T- test.** The mean scores of the categories, based on exam and stress, were compared using an independent *t*-test (for two groups) . $p<0.05$ was considered statistically significant. Using binary logistic regression, we performed an adjusted analysis.

## RESULT

The survey has received about 304 replies. For the purposes of the survey study, the minimum age start for students was set at 18 to 25 years old. 50 percent of the participants are localities and 50 percent are hostilities. All 304 students are from Parul university, Vadodara, india. The statistics showed that, although 148 participants (49%) did not experience any dietary modifications during the exam, 156 participants (51%) did experience changes in their diet. Based on the data, we deduce that 50% of individuals had diet modifications across for many days during the examination days. (table 1). There is a correlation of ongoing exam and diet pattern change (r=-0.82), based on (n=303) there is a slight loss of appetite as exams are going on. Based on which conclusion is that changes in dietary patterns due to exams pressure. The examination increases the stress which might also be influencing the diet change.

The correlation between change in eating with sleep pattern and examination days (r=-0.36) , the number of observations of caffeine product (n=303) there is a weak negative relation between students who have currently ongoing examinations and there sleeping & eating pattern are likely to change this line with understanding that examination stress increases and eating pattern , sleep duration decrease. Out of all the individuals, 78(25.65%) experienced regular changes in their eating and sleep patterns, 87(29%) experienced stressful dietary changes with sleep pattern, 51(27%) experienced insomnia (a condition characterized by poor sleep), and 88(29%) experienced variations in their eating and sleep patterns. According to the data,30%participant do feel changes in dietary habit and sleep pattern. (table 2). The correlation of diet changes and feeling nervous due to stress (r = 2.31), the number of observations of diet meal (n=303). There is a strong positive relationship between prefer diet changes during stress time. So, it clearly says about the increased ratio of diet changes with stress.

The data of correlation was based on eating with sleep pattern changes and examination (r=-0.36) , the number of observations of caffeine product (n=303) there is a weak negative relationship between increased stress of exam days and changes in eating and sleeping behaviour. The correlation of eating with sleep pattern changes and total stress (r=0.02) , the number of observations of caffeine product (n=303) there is a linear relationship. So, it is a clear observation that caffeine product consumption increases with changes in eating and sleep pattern. Out of all the participants in the questionnaire, 49 (16%) slept for at least seven hours, 69 (23%) for a maximum of six hours, 104 (34%) for more than four hours, and 82 (27%) for more than five hours.(table 3). The correlation of total stress and sleep schedule during exam (r=-1.52), the number of observations of total stress (n=303). There is an extremely strong negative relationship between increased stress level and decreased hours of sleep during exam days. which implies getting less sleep during exam day. Pearson correlation of feeling dizzy and sleepy during exam days (r=0.85), the number of observations of feeling dizzy (n=303). There is a moderate positive relationship between stress level with hours of sleep during exam days.

## CONCLUSION

The correlation between exam days and diet pattern change(r=-0.82), soft drink and mid-night food craving (r=-0.38), dietary habits with sleep pattern (r=0.36), current exam and cooking preference by PG students(r=--0.13), soft drink and feeling nervous (r=-0.41),stress and sleep schedule during exam (r=-1.52) and exam and caffeine product (r=-0.36) based on Total no of population (n=303) Stress and alterations in eating and sleeping patterns are strongly correlated negatively. The evidence suggests that dietary modifications may disrupt sleep patterns and that studying for college exams is the root cause of these disruptions. The correlation between diet changes and feeling nervous due to stress(r = 2.31),sleep duration during exam days and feeling lethargic(r=0.85 ),soft drink and eating pattern during exam days (r=0.23), eating pattern and Local lite food preference by PG students (r=0.20),currently pursuing and preference of food by PG students (r=0.21),mess food and exam ( r=0.33)based on no.of population (n=303)there is a strong positive correlation between exam and eating pattern, sleep duration. Exams associated with lethargy, dietary changes, and the preferred cooking methods of PG students are positively correlated.

The correlation between exam and gender, age, caffeine product and soft drink and ongoing exam (r=0.10) based on no. of correlation (n=303) there is no specific relation between hot and cold beverages with exam and nor with age and gender. Null hypothesis: - There is no correlation between examination stress and changes in eating behaviour among 18 to 25 y/o students of Parul university. Alternative hypothesis: -There is correlation between examination stress and changes in eating behaviours among 18 to 25 y/o students of Parul university.

According to this finding, the null hypothesis is rejected since statistical evidence supports the alternative hypothesis, which demonstrates a substantial relationship between exam stress and modifications in sleeping and eating habits.
In Conclusion, Stress and alterations in eating and sleeping patterns are strongly correlated negatively. The evidence suggests that dietary modifications may disrupt sleep patterns and that studying for college exams is the root cause of these disruptions. Positive correlations have been shown between exams linked to sluggishness, dietary modifications, and PG students' favourite cooking techniques. There is no particular correlation between exam scores and gender or age.

This result means that the alternative hypothesis—which shows a strong correlation between exam stress and changes in sleeping and eating patterns—is statistically supported, rejecting the null hypothesis.

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**Conflict of interest**

There is no conflict of interest between author regarding the paper publishing.

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**Table 1 Diet changes during exam day**

| Change in diet | Response | Percentage |
|---|---|---|
| Yes | 156 | 51.31% |
| No | 147 | 48.68% |
| Total | 303 | 100% |
| Examination | | |
| Yes | 100 | 32.89% |
| No | 184 | 60.5% |
| May be | 19 | 7% |
| Total | 303 | 100% |

**Table 2. Responding about their changes in eating and sleep pattern**

| Eat and sleep pattern | Respondent | Percentage |
|---|---|---|
| Regularly | 78 | 25.65% |
| Stressful | 87 | 28.61% |
| Insomnia (related to poor sleep) | 51 | 16.77% |
| Most of the time | 87 | 28.94% |
| Total | 303 | 100% |

**Table 3 Sleep cycle during exam days**

| Sleep during exam days | Respondent | Percentage |
|---|---|---|
| At least 7 hours | 49 | 16.11% |
| 6 hours | 69 | 22.69% |
| 4 hours to 5 hours | 104 | 34.21% |
| less than 5 hours | 81 | 26.97% |
| Total | 303 | 100% |

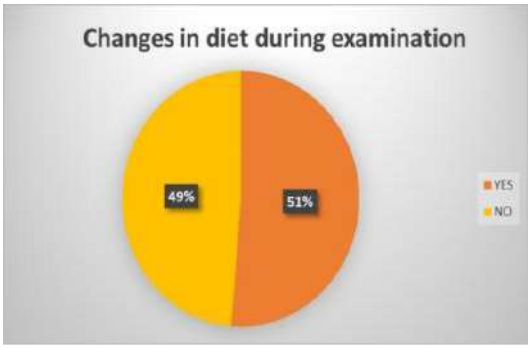


**Figure 1Diet changes during exam day**

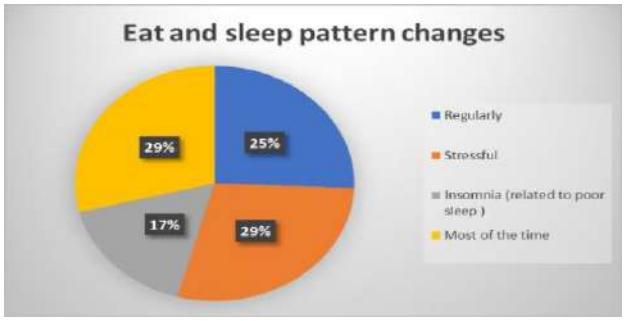


**Figure 2 Responding about their changes in eating and sleep pattern**

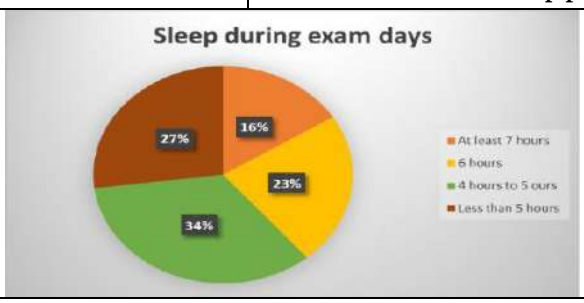


**Figure 3 Sleep cycle during exam days**

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**RESEARCH ARTICLE**

# Using Fuzzy Mathematical Modelling to Study Student's Mental Health During Exam Preparations

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**ABSTRACT**

Today, people are mostly affected by stress due to work, financial problems, personal relationships, parenting, daily life and busyness, personality, and resources. But we didn't note that students were also affected by stress in their exam preparations. Several studies say that stress leads to anxiety and depression. We all know health is wealth, so health is made up of three major categories: physical health, social health, and mental health. During semester exam preparation, students' anxiety to face exams increases, which leads to stress and depression. In this paper, we study students' mental health during exam preparations using a fuzzy inference system. We split the analysis period into three months: the first 2$^{nd}$ month, the next 4$^{th}$ month, and the 6$^{th}$ month. And also, we suggest some ideas to overcome the stress, anxiety, and depression during exam preparations and achieve good marks.

**Keywords:** Fuzzy Inference System, Stress, Anxiety, Depression, Health, Trapezoidal Fuzzy Number,

## INTRODUCTION

In today's world, mostly people survive among stress, anxiety, and depressed situations, like stress in the workplace, financial crises, problems in personal relationships, difficulties in parenting, daily life and busyness, personality, and resources. Among these problems, we forget to note some issues, namely that students are also facing stress, anxiety, and depression during their exam time, like preparing for the exams, writing the exams, and waiting for the exam results, which lead to stress and depression. If they score good marks, there is no problem; if they score bad or average marks, then it may lead them to stress and depression by thinking about their future. Some students survive in these situations, but some students make wrong decisions like suicide or self-harm.

In this paper, we discuss students' mental health during exam days and their stress, anxiety, and depression levels during exam preparation, writing exams, waiting for the results, and due to score cards. The study was analysed using a fuzzy inference system. The study period was split into three months, i.e., the first 2nd month, the next 4th month, and the 6th month. And also giving ideas to overcome the stress, anxiety, and depression during exam preparations and achieve good marks.

**Causes and Effects of Stress, Anxiety, and Depression:**

Due to stress, anxiety, and depression, human's mental, physical, and social health are affected. Most commonly, physical health problems are noted easily by symptoms like headaches, high blood pressure, weight gain, weight loss, fatigue, chest pain, muscle tightening, sweating, dizziness, and insomnia. Similar symptoms for mental health problems include being unable to relax, difficulty concentrating, avoiding particular situations and objects, excessive worrying, feeling overly anxious to fit in, troubles in making decisions, feeling very sad or hopeless, thoughts of self-harm or suicide, irritability, changes in eating behaviour, sleep changes, and loss of interest in hobbies and activities.

Diseases led by stress, anxiety, and depression are cardiovascular disease, upper respiratory disease, human immunodeficiency virus, inflammation of the immune system, and inflammation of cytokine production. Treatment and medication for these problems include yoga, exercise, meditation, and simply doing physical activity. Seek help from proper mentors and doctors to recover from the stress and depression. Stress, anxiety, and depression are rated by scales; namely, for the stress scale named the Perceived Stress Scale (PSS), the ratings are 0 – never, 1 – almost never, 2 – sometimes, 3 – fairly often, and 4 – very often. For the anxiety scale named the Hamilton Anxiety Scale (HAM-A), the ratings are 0 – not present, 1 – mild, 2 – moderate, 3 – severe, 4 – very severe. For the depression scale named the Hamilton Depression Rating Scale (HDRS), just as for anxiety, the ratings are: 0 – not present, 1 – mild, 2 – moderate, 3 – severe, 4 – very severe.

**Fuzzy Inference System:**

Fuzzy inference is the process of formulating the mapping from a given input to an output using fuzzy logic. The mapping then provides a basis from which decisions can be made, or patterns discerned. The process of fuzzy inference involves all the pieces that are described in Membership Functions, Logical Operations, and If-Then Rules [1].

**Two months analysis**

For the first two months, the range of stress, anxiety, and depression will be [2–34]. For normal, the ranges shown as trapezoidal membership functions are stress [6 8 10 16], anxiety [1 2 4 9], and depression [1 1 2 4]. For mild, the ranges shown as trapezoidal membership functions are stress [9.8 12.9 15 22.3], anxiety [2 2 8 20], and depression [6 8 10 16]. For moderate, the ranges shown as trapezoidal membership functions are stress [5 10 19 42], anxiety [6 8 10 16], and depression [5 8 14 29]. For severe, the ranges shown as trapezoidal membership functions are stress [8 15 34 47], anxiety [9.8 12.9 15 22.3], and depression [9 15 21 39]. For extremely severe, the ranges shown as trapezoidal membership functions are stress [14 20 34 68], anxiety [9 10 20 41], and depression [10 28 32 42].

The above table shows that ranges and ratings of stress, anxiety and depression for two months. The output range and rating for two months combined stress, anxiety, and depression levels shown as trapezoidal membership functions are for normal [2 3.9 5.3 10], mild [5 7 11 21], moderate [6 13.9 14.3 23], severe [15 20.7 22 25.1], and extremely severe [18 27.3 30 33.9].

**Four months analysis**

For the next fourth month, the range of stress, anxiety, and depression will be [5-45]. For normal, the ranges shown as trapezoidal membership functions are stress [3 7 12 26], anxiety [2 3 5 10], and depression [1 5 6 12]. For mild, the ranges shown as trapezoidal membership functions are stress [7 16.2 16.8 24.8], anxiety [4 5 8.4 16.1], and depression [6 12.1 14 16.3]. For moderate, the ranges shown as trapezoidal membership functions are stress [9 15 21 39], anxiety [3 7 12 26], and depression [10 15.9 18.2 19.5]. For severe, the ranges shown as trapezoidal membership functions are

stress [10 28 32 42], anxiety [9 13 17 29], and depression [9 17 23 43]. For extremely severe, the ranges shown as trapezoidal membership functions are stress [21 33 45 81], anxiety [5 12 36 43], and depression [7 22 30 61]. The above table shows that ranges and ratings of stress, anxiety and depression for fourth month. The output range and rating for fourth monthcombined stress, anxiety, and depression levels shown as trapezoidal membership functions are for normal [5.6 7.7 8.5 9], mild [6 12.3 14.9 16], moderate [10 16.3 18.9 20], severe [13.2 18.8 22.7 36.1], and extremely severe [20 33 35 44].

**Six months analysis**

For the next sixth month, the range of stress, anxiety, and depression will be [7-57]. For normal, the ranges shown as trapezoidal membership functions are stress [5 8 14 29], anxiety [2 4 7 14], and depression [3 7 9 17]. For mild, the ranges shown as trapezoidal membership functions are stress [7 18 23 24], anxiety [3 7 9 17], and depression [8 11 13 20]. For moderate, the ranges shown as trapezoidal membership functions are stress [6 19 25 50], anxiety [5 8 14 29], and depression [9 10 20 41]. For severe, the ranges shown as trapezoidal membership functions are stress [12 24 33 63], anxiety [5 10 19 42], and depression [9 12 27 60]. For extremely severe, the ranges shown as trapezoidal membership functions are stress [24 57 64 83], anxiety [8 26 39 83], and depression [18 36 48 90]. The above table shows that ranges and ratings of stress, anxiety and depression for sixth month. The output range and rating for sixth month combined stress, anxiety, and depression levels shown as trapezoidal membership functions are for normal [9 9 10 12], mild [9.2 10 12 22], moderate [10 13.7 14 17.1], severe [12 26.3 26.9 40], and extremely severe [38 48 52 54]. The above graph shows the overall outcome of stress, anxiety, and depression levels throughout the six months. Here we can see for the first 2 months the range between [2–34], next for 4 months the range between [5–45], and after six months the range between [7–57].

## CONCLUSION

From the past six-months analysis, split into two months, we conclude that comparing the first two months to the last two months, which are the fifth and sixth months, stress, anxiety, and depression are higher for students. Because the last two months are exam months. This analysis was conducted with the help of a fuzzy inference system using MATLAB software. The analysis is explained with the help of individual plots for stress, anxiety, and depression levels at 2 months, 4 months, and 6 months. The overall range for the fuzzy inference system is taken as [1–57], and the table values for stress, anxiety, and depression are calculated for those particular months.

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**Table 1: shows that ranges and ratings of stress, anxiety and depression.**

| Stress | Anxiety | Depression | Ratings |
|---|---|---|---|
| 0-14 | 0-7 | 0-9 | Normal |
| 15-18 | 8-9 | 10-13 | Mild |
| 19-25 | 10-14 | 14-20 | Moderate |
| 26-33 | 15-19 | 21-27 | Severe |
| 34+ | 20+ | 28+ | Extremely Severe |

**Table 2: shows that ranges and ratings of stress, anxiety and depression for two months.**

| Stress | Anxiety | Depression | Ratings |
|---|---|---|---|
| 10 | 4 | 2 | Normal |
| 15 | 8 | 10 | Mild |
| 19 | 10 | 14 | Moderate |
| 26 | 15 | 21 | Severe |
| 34 | 20 | 28 | Extremely Severe |

**Table – 3: shows that ranges and ratings of stress, anxiety and depression for fourth month**

| Stress | Anxiety | Depression | Ratings |
|---|---|---|---|
| 12 | 5 | 6 | Normal |
| 16.2 | 8.4 | 12.1 | Mild |
| 21 | 12 | 15.9 | Moderate |
| 28 | 17 | 23 | Severe |
| 45 | 24 | 30 | Extremely Severe |

**Table – 4: shows that ranges and ratings of stress, anxiety and depression for sixth month.**

| Stress | Anxiety | Depression | Ratings |
|---|---|---|---|
| 14 | 7 | 9 | Normal |
| 18 | 9 | 13 | Mild |
| 25 | 14 | 20 | Moderate |
| 33 | 19 | 27 | Severe |
| 57 | 39 | 48 | Extremely Severe |

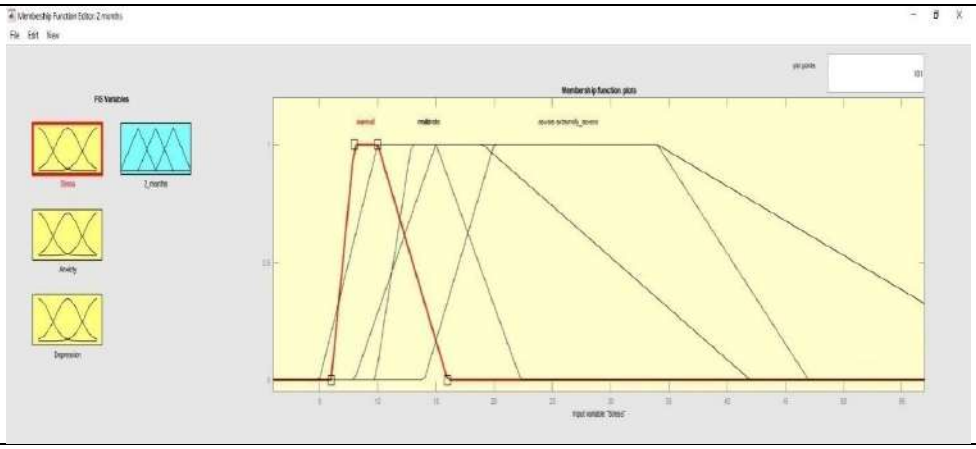


**Figure – 1 Plots representing stress level for two months**

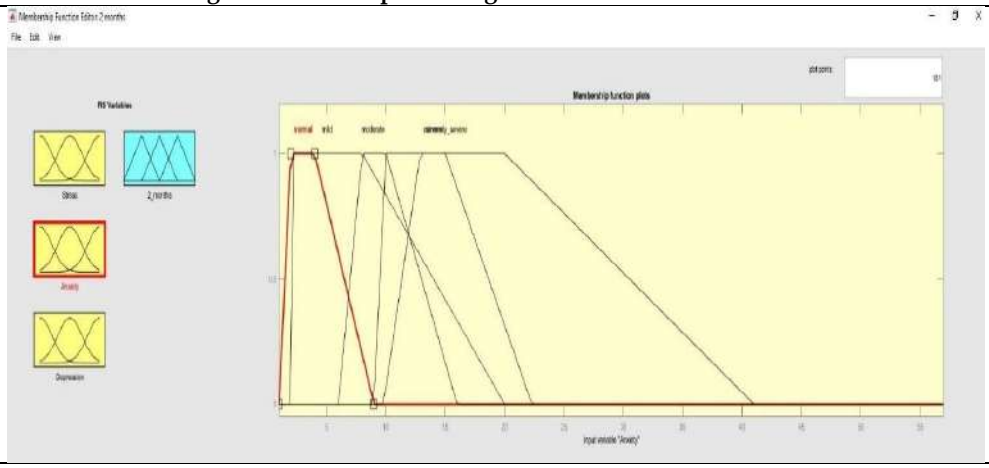


**Figure – 2 Plots representing of anxiety level for two months**

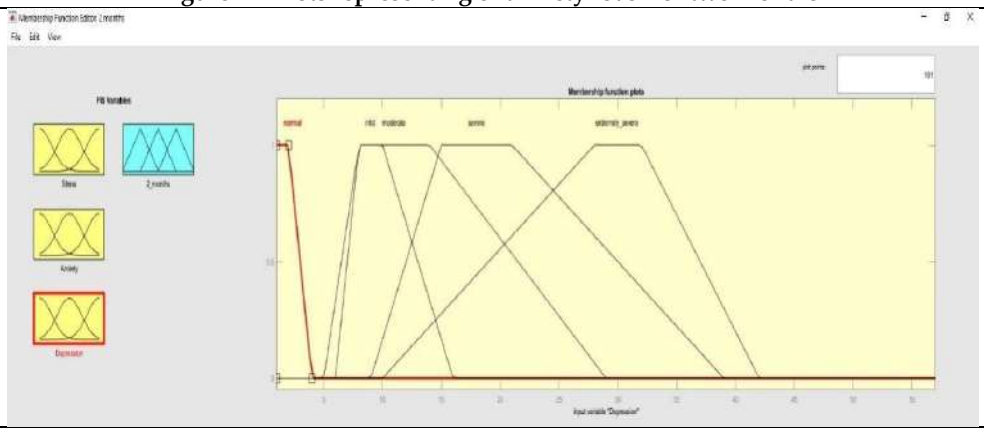


**Figure – 3 Plots representing depression level for two months**

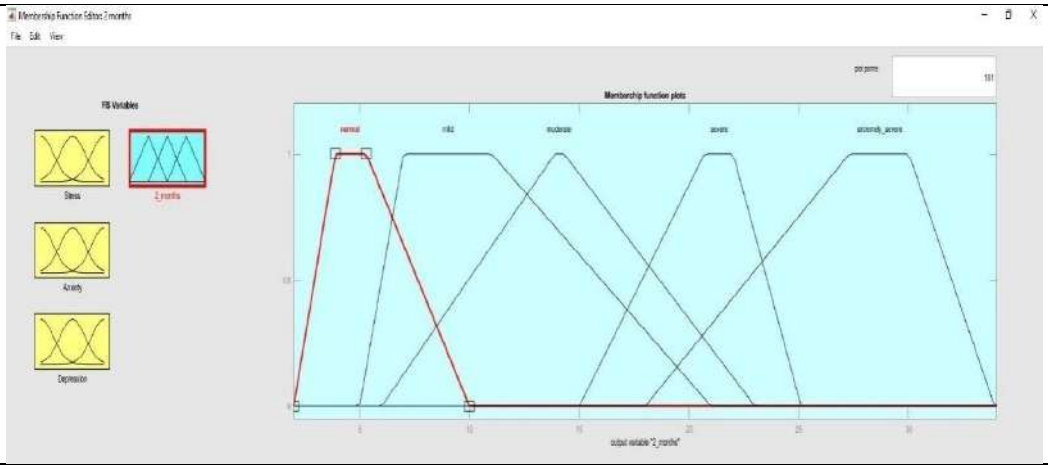


**Figure – 4 Plots representing output for two months**

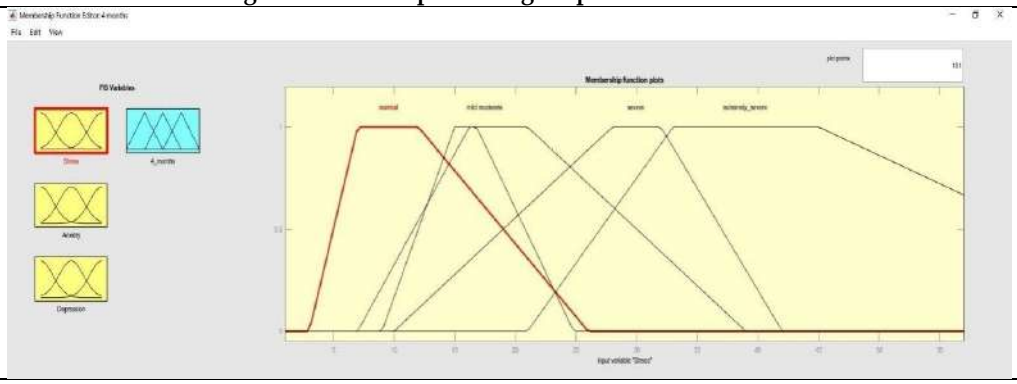


**Figure – 5 Plots representing stress level for fourth month**

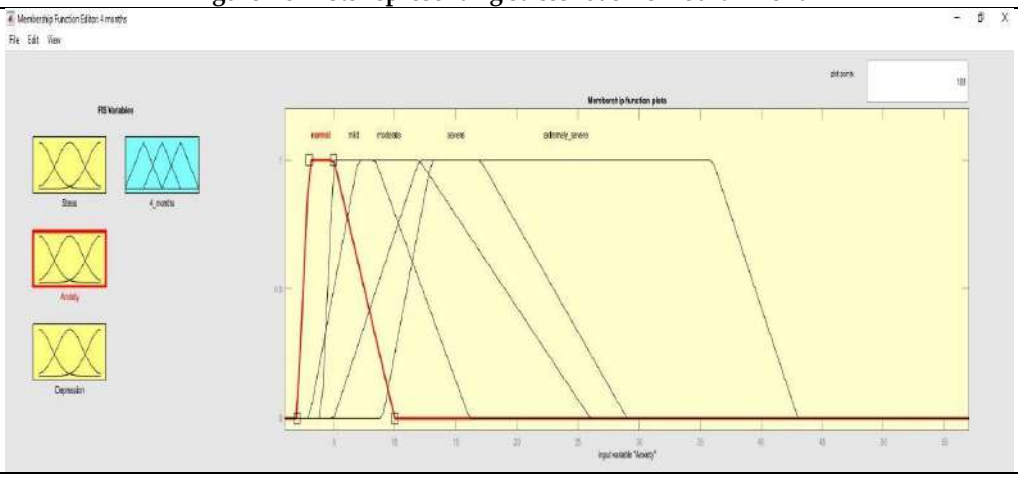


**Figure – 6 Plots representing anxiety level for fourth month**

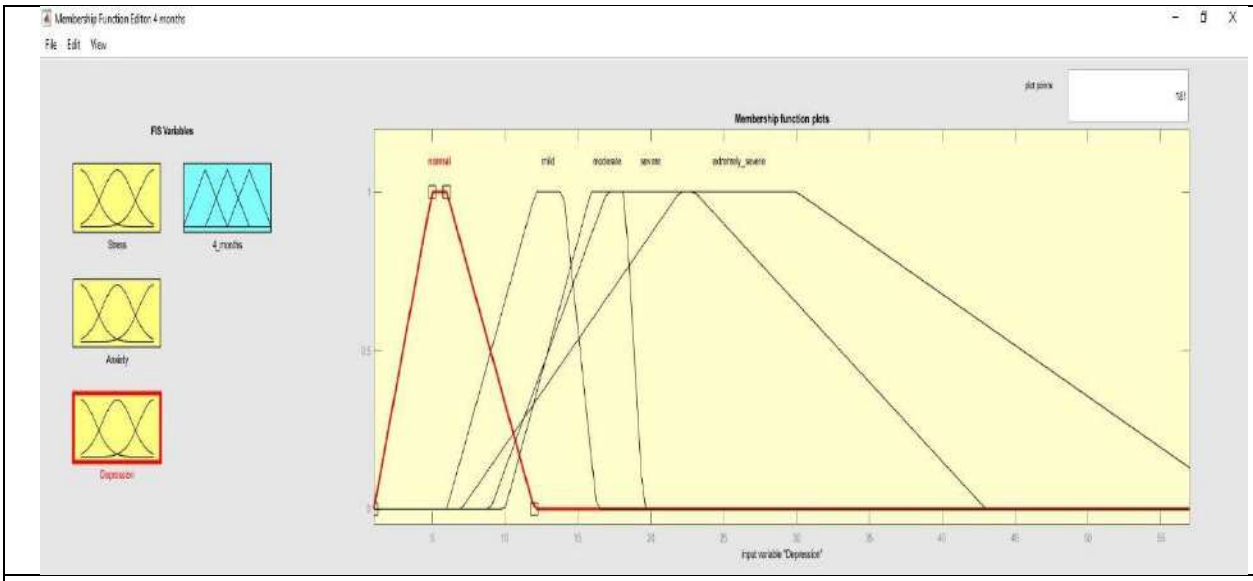


**Figure – 7 Plots representing depression level for fourth month**

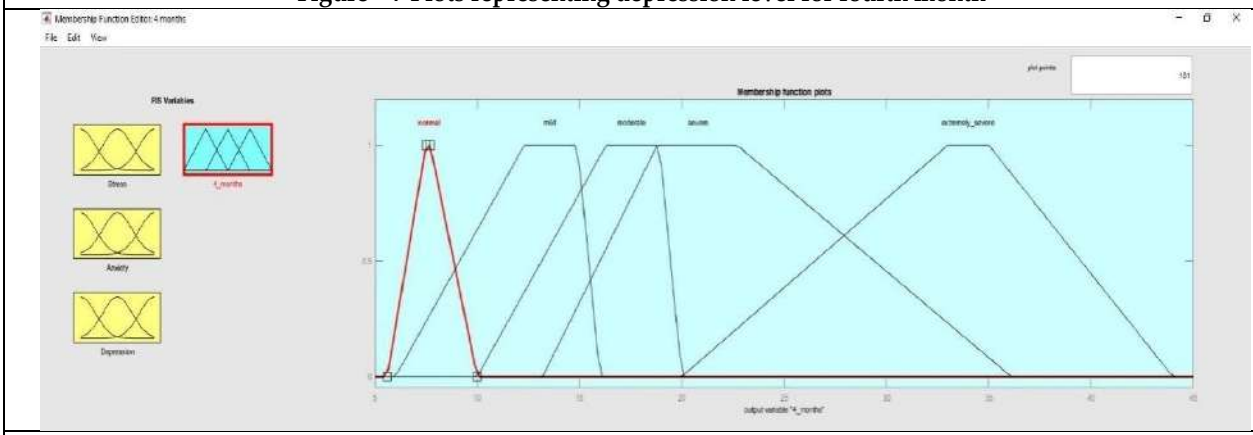


**Figure – 8 Plots representing output for fourth month**

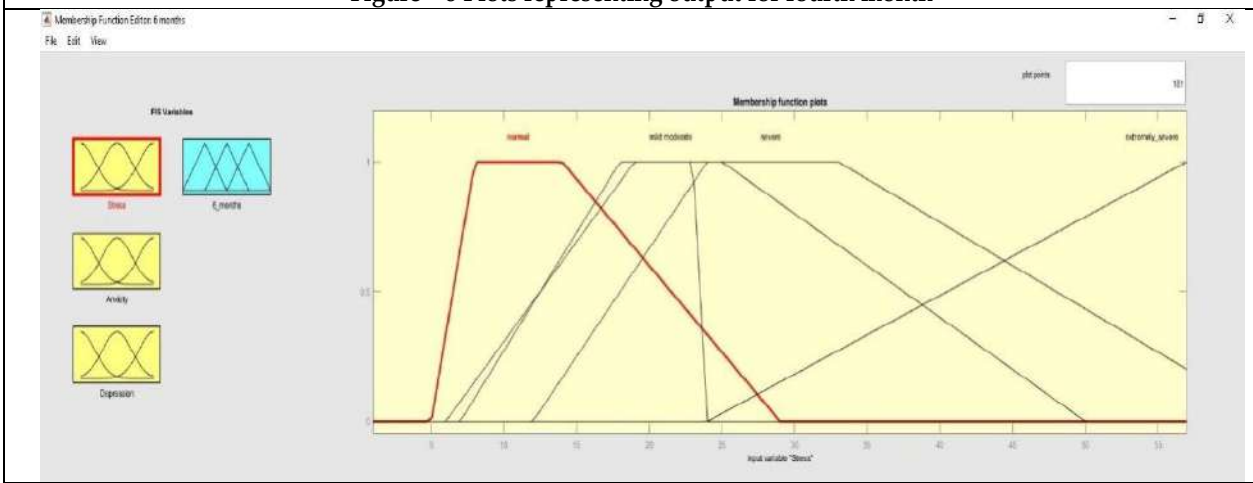


**Figure – 9 Plots representing stress level for sixth month**

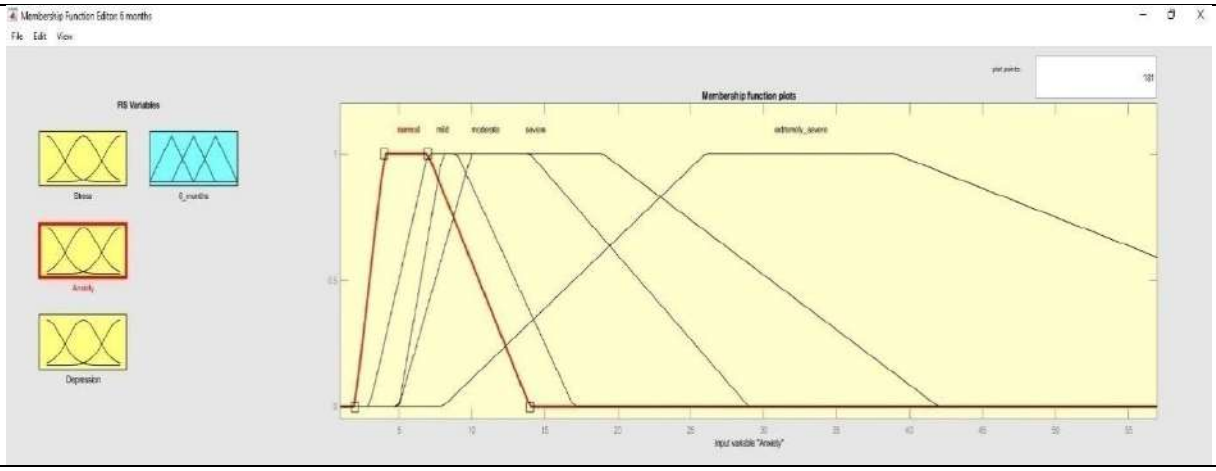


**Figure – 10 Plots representing anxiety level for sixth month**

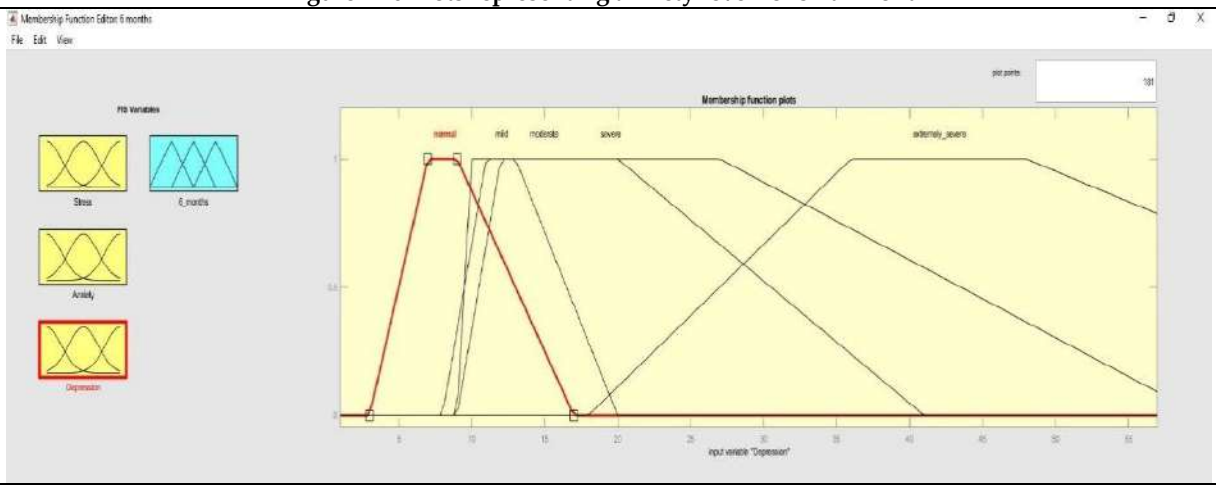


**Figure – 11 Plots representing depression level for sixth month**

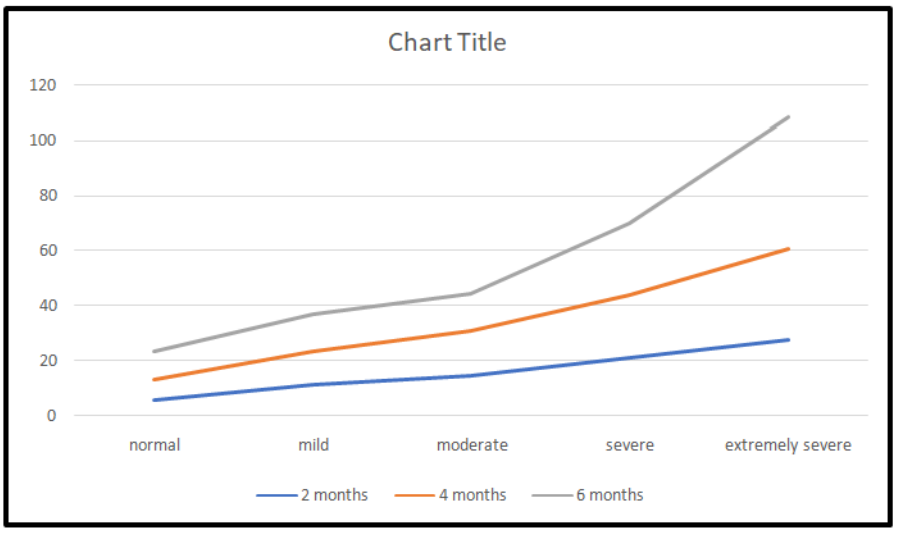


**Figure – 12 Graph representing overall outcome of stress, anxiety and depression level**

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**RESEARCH ARTICLE**

# Industrial Plant Automation Management Best Practices for Great Outcomes

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**ABSTRACT**

In the current era of smart homes and smart grids, advanced technological solutions that allow automation of household functions are rapidly evolving. Today's smart homes have a wide variety of technologies and applications. These are key in improving automation, monitoring and remote control capabilities by initiating communication between home appliances and their customers. In this article, examples of automation that focuses on energy efficiency, environment, automation are explained. Technical descriptions of the system are provided along with the advantages and disadvantages of each technology and product currently on the market. The role of consumers is also highlighted along with the barriers, obstacles, rewards and future developments in technology. The author has tried his best to justify Management best practices for great outcomes.

**Keywords**—Smart Grid, Home Automation, Energy Management System, Plant Automation, Programmable logic Controller (PLC), Supervisory Control and Data Acquisition (SCADA) .

## INTRODUCTION

Automation is a crucial idea for the industry 4.0 and is becoming more and more valuable to industrial businesses [1]. The global industrial automation market was valued at 157.04 billion dollars in 2018 and is projected to grow to

296.70 billion dollars in 2026, nearly twice as much as it was a year earlier, according to the publication Fortune Business Insights. Organizations are drawn to automation-facilitating technologies like blockchain, artificial intelligence (AI), and the Internet of Things (IoT) because of their advantages [1]. It offers exceptional benefits in numerous areas, but most notably in terms of time and money savings. Businesses today must use industrial automation systems to complete industrial automation processes in order to benefit from these advantages. Industrial automation systems are those that use computers to control and monitor machines, processes, or other devices. Usually, these systems are employed to perform recurring duties or operations. Their aim is to operate independently, reducing and increasing the quantity of human labour needed in the industry. These systems take the place of the monotonous, mechanical jobs that are typically completed by a single person, as well as the choices that person makes during the manufacturing process. Strong machinery and logical programming commands are used to accomplish this.

Various forms of automation are available to businesses that decide to begin an industrial automation process, based on factors such as production volume, product range, and varying degrees of adoption. The phrase "home automation" or "fixed automation" refers to an automation procedure in which a machine or robot is designed to carry out a single, highly repetitive task for a high output volume [2]. These processes are fast, accurate, safe and have high production rates, however they are fixed and cannot be altered. The advantage of this kind of automation management offers the sectors are best suited for operations with a high volume of load and little work variability. The most cost-effective kind of automation is this one.

Soft automation refers to equipment that can adapt to a wide variety of product configurations. The process is managed by a computer that can be configured to change how it operates depending on the product. When a sector produces a variety of goods that call for distinct and varied configurations, it is quite helpful. Compared to hard automation, this kind of automation is more expensive and runs more slowly. It is especially appropriate for establishments that produce a small number of comparable goods in small quantities using batches. Programmable automation and flexible automation are the two categories into which this type of automation falls. Programmable automation category includes automated industrial processes that are managed by a program that is made up of a series of coded instructions that the system must decipher. Because of the system's great flexibility, new products can be made using these processes and new software. The equipment can be configured and adjusted in terms of both software and hardware. It is recommended to employ this kind of automation exclusively in low-production industries because it is difficult and time-consuming to reprogramme a machine to accommodate new products. As such, the processes are finished in batches.

A machine can be quickly configured to fit a new product thanks to flexible automation. This minimizes the amount of time lost when switching from one item to another in terms of configuration. Because this type of automation removes the need to waste time reprogramming the system or altering the physical configuration of the machines, there is no need to produce the products in separate batches. This type of automation is recommended for medium-sized productions. Flexible automation is used to enable continuous production that can be adjusted to the different product types that an industrial plant may encounter. Although automation removes the need for manual labour in factories, operators are still needed to keep an eye on equipment and perform maintenance. On the other hand, human intervention in the process is no longer necessary when using an industrial automation system. One of the biggest issues facing one is boosting energy security and accessibility in light of diminishing energy resources, particularly in emerging economies. An essential agenda item is producing electricity effectively and efficiently, in addition to managing the energy resources that are currently available. The construction of large power plants using conventional energy sources must be complemented, particularly with small-scale power generation distributed from renewable energy sources. Distributed power sources (DERs) can be connected to the grid with additional infrastructure and financial outlays, but these technologies minimize transmission and distribution (T&D) losses and do away with the need for a costly transmission system. Adopting a methodical strategy that views output and related load as a subsystem or "micro-grid" is a smart way to realize the growing potential of distributed generation[3].

One common type of small integrated circuit is a microcontroller. A microcontroller is a small integrated circuit device that is usually used to control a single function inside another device, like a television, automobile, or home appliance. It's also typical practice to use several microcontrollers for different tasks. A car, for instance, would have different microcontrollers for the powered windows, the auto-braking system, and the speedometer. In contrast, PLCs are made up of an external programming device, a power supply, an I/O module, and a processor. The PLC's brain, or processor, uses data from connected input devices, like switches, sensors, and thermometers, to carry out preprogrammed control operations. For instance, After analysing thermometer-transmitted low-temperature data, the PLC's processor will decide on the best course of action and send a command to activate a heating element. Field devices are physically connected to the PLC through its I/O modules, which are also in charge of sending commands to output devices and bringing in data for the processor. It is possible to combine or separate analog or digital I/O modules based on the requirements of the application.

The PLC program is written and downloaded to the controller using the external programming device, which is usually a desktop or laptop computer. Ladder logic, function block diagrams, structured text, instruction lists, and sequential flow charts are a few of the numerous PLC programming methods that are available [4]. A microcontroller is a small integrated circuit device that is typically used to control a single function in another device, such as a television, car, or household appliance. Using several microcontrollers to carry out distinct tasks is also typical. A microcontroller is used in the car, for instance, to control the speedometer, the auto-braking system, and the powered windows. comprises an external programming device, a power supply, an I/O module, and a PLC processor.

The PLC's brain, or processor, uses data from connected input devices like switches, sensors, and thermometers to carry out preprogrammed control operations. For instance, when the PLC receives low-temperature data from the thermistor, its processor evaluates the information, chooses the proper action, and then initiates the command to activate the heating element. The I/O modules of the PLC are physically attached to the field devices, and they transmit commands to the output devices and receive data as input for the processor. Analog or digital I/O modules can be mixed and matched to suit a given application. Writing programs for desktop or laptop computers and PLCs, as well as downloading them to the controller, is typically done via an external programming device. Among the PLC programming techniques are ladder logic, function block diagrams, structured text, instruction lists, and sequential flow charts [4], [5].

Numerous new opportunities for efficiency gains have arisen as a result of the smart grid initiative, which aims to modernize the infrastructure of electric utilities. An effective method for working remotely and keeping an eye on renewable energy sources is to use a supervisory control and data acquisition (SCADA) system. SCADA systems are extensively employed in numerous industrial applications, and they have contributed to these systems' increased efficiency. In today's modern communication age, the smart grid initiative, which aims to integrate both function and architecture, has made tremendous strides in modernizing and expanding electric utility infrastructure. With this progress come organizational, socioeconomic, technological and cyber security challenges. The scope and magnitude of those challenges have increased significantly and many regulatory bodies have taken steps to align their standards and regulations with these new challenges.

K. Sayed and H.A. Gabbar received an overview of the process for gathering and using data in SCADA systems, development, and power systems. Along with the standard remote terminal units (RTUs), it also includes master stations, data concentrators, outstation hardware, and the newest intelligent electronic devices (IEDs). The fundamentals of Power System Supervisory Control and Data Acquisition (SCADA) and potential application functions increased the viability of smart grids and encouraged more individuals to participate in their development [6]. Research on various automation methodologies, including PLC, SCADA, and DCS (distributed control systems), and their benefits and drawbacks, has been conducted by Hudedmani*, Hittalamani Raghavendra, Umayal R M, and Shiva Kumar Kabberalli. The mention table below provides a summary of the findings[7].

ER Alphonsus, MO Abdullah PLC provided an overview of its various applications such as hardware, programs and water and wastewater management control, solar-tracking systems, wind power, photovoltaic applications. Control of heating, ventilation, and air conditioning (HVAC), research, instruction, and training, as well as plant control and monitoring and other uses [8]. An air control system powers the packaging system's assembly and global operation. Pneumatic was only ever used manually in the past; nowadays, PLC is used to automate the process [9].

Factories in Industry 4.0 use logic controllers to monitor system status, decide on actions based on customized programs, and take necessary action. Controllers use high-speed computations outlined in programs (control logic) to enforce control in real time, and they output the results as output signals to actuators and other devices. Figure 1 depicts the specifics of the Smart Control Box (SCB) architecture. A single board PC and an MCU (Micro Control Unit) make up a SCB. The MCU receives the input signals from the PLC. For edge computing and data storage, signals are sent to a single board PC via wired or wireless communication. The MCU responds to the PLC's decision command following the margin analysis. In order to close the gap between Industry 3.0 and Industry 4.0, this research aims to introduce a SCB. Installing intelligence on a single board computer and connecting the Internet of Things to the PLC with the MCU [10].

The PLC community has responded to evolving needs by developing new programming languages, implementing special priority scan cycles, and developing specialized I/O hardware (hidden networking) through PLC programmers. Memory and processing speed have also improved dramatically over time. interactions between supervisory layer systems like SCADA, distribution control systems, or human-machine interfaces and field devices like sensors and actuators. Enterprise resource planning and implementation systems process the data that is received in both the upper and lower layers [11]. As depicted in Figure 2, PLCs are centralized controllers that interact with sensors and actuators as a component of a conventional automation pyramid. All connected devices are perceived as I/O devices, with no awareness of any other behaviour besides the inherent values and complexity of each device. shared and taken in. Decentralized control and greater use of intelligence are predicted by trends like Industry 4.0 [11].

Effective data transmission in communication networks is necessary for the automation, monitoring, and tracking of technical activities in both industrial and non-industrial settings. This information is related to duties like receiving, logging, measuring, and displaying data. The transferred data also includes control codes. Digital communications are used by control units, computers, sensors, actuators, human-machine interfaces (HMI), and networks with various topologies to transfer operational data and command signals [12].

**Plant Automation Case Study**

Power plants and other businesses that use boiler equipment need to constantly monitor and inspect it on a regular basis. In essence, the boiling section of the boiler which generates hot water for the production of steam must be run concurrently with other parts of the boiler, including the core section, which includes the boiler drum. Not enough steam is produced when the water level is too high. In order to automate the system and reduce human error, it is crucial to measure and maintain the levels of steam and drum. It is essential to keep the water level in the drum precisely under control. The reduction of human error rates and the enhancement of control and supervision over plant or process operations can be accomplished through the development of PLC and SCADA systems, which can be employed for the entire plant and running. possesses the ability to be applied to process monitoring and provide the required data in response to process changes.

**PLC Temperature Control Programming**

Temperature in each heater is measured by three thermostats. Different set points with different ranges is with all three heaters. The table shows the temperature ranges.

1. all three heaters (H1, H2, H3) must be on when a temperature goes below 55° C,
2. The optimal operating temperature range for both heaters (H2, H3) is between 55° and 60° C.

3. The operating temperature range for the heater (H3) is 60° to 65° C.
4. Every heater that is over 70°C needs to be in the OFF position and have a safety shutoff (relay CR1) in case something goes wrong.
5. enables and disables the master switch system.

**Solution :**

- There are four thermostats; if the set point is not reached, assume they are in the NC (usually closed) position.
- Allow the control relay (CR1) to function as a shut-off for safety.
- Master switch: Turn on switch NO, which is normally open, and turn off switch NC.

The temperature ranges indicated by the thermostats' temperature values (TS1, TS2, TS3, TS4) are displayed in Table 2. In addition, the heaters (H1, H2, and H3) are either in the on or off position based on the temperature reading.

**First step**

Its two buttons are for start (no default contact) and stop (default NC contact). Depending on the state of the thermostats, Relay CR1 regulates the heaters. All heaters are turned off if the TS4 is energized (TS4 contact changes from NC to NO). The thermostat is connected between the TS4 STOP and the relay. There is no way to lock or hold the START command when Relay CR1 is introduced to the START button.

**Second step**

Heaters (H1, H2, and H3) can be controlled by relay CR1 in the absence of contact thermostats (TS1, TS2, TS3).After the START command is issued, this NO contact closes (becomes an NC contact). All heaters are on when the temperature drops below 55°C because TS1, TS2, and TS3 are closed. Heater H1 is turned off when the temperature is between 55° and 60° C because TS1 is open. TS2 will then open as well, turning off the heater H2 if the temperature is between 60° and 65° C. When the temperature is between 65° and 70° C, TS3 also opens, which turns off the heater H3. To avoid overheatingor any thermostat malfunction, there is a safety shutoff available. All heaters will be turned off by the TS4 powering the relay and reducing power if the temperature rises above 70°C.

Note: In this case, the contactors or relays that we are turning on are the heaters H1, H2, and H3. As a result, the electric heater feeder circuit (MCC) is not connected to the contact of these relays. These signals regulate these electrical feeder circuits, and the heaters are turned on or off in accordance.

**Boiler Control**

The heating elements are gradually heated and gradually turned off when the boiler is turned off to prevent excessive electricity consumption at the boiler's startup. Figure 5 depicts this working principle. The first heating element (S1) activation is powered by the ON (MA) button. The activation of the second element (S2) occurs following the time delay T. The time-delay T is again stopped by the third element (S3) and the fourth element (S4) following the same time delay. S1's Off (AR) button. After a little delay, the remaining three components progressively become inactive.

**Green House Ventilation Panes**

It is far more difficult to ventilate and cool greenhouses than it is to heat them. The temperature can always be adjusted by adding more heat, but in severe weather, it is challenging to lower the temperature. Greenhouse ventilation, which provides $CO_2$ and controls temperature and humidity, is necessary for crop production. The two most popular ventilation methods utilized in greenhouse production systems are mechanical and natural ventilation systems. The normal airflow produced by air pressure or the gradient created by temperature differences between the rising area and the rest of the greenhouse are what allow for natural ventilation.

The process of moving air through fans, known as mechanical ventilation, involves bringing air into the growing area through carefully regulated greenhouse entrances and releasing it through the fan assembly. Good mechanical ventilation systems must be flexible enough to accommodate changing inlet shapes. Thermostats and, in certain situations, moisture-sensing devices regulate fan ventilation, whereas relative humidity is the primary factor in the control of disease [14]. The most basic and conventional environmental control system for tropical greenhouses uses sensors to monitor all significant environmental factors that affect crop growth, both inside and outside the greenhouse. The controller then receives the sensor data. The environmental parameters are managed by preprogrammed commands and related actuators, including fans, coolers, fogging systems, lighting systems, and $CO_2$ sprayers [15].

**Mechanical Ventilation**

An intelligently designed inlet allows fan ventilation systems to efficiently control temperature all year round. The most desirable feature is the ability to easily automate the entire system. With the introduction of computer-based control, this is becoming more and more the case. This feature will be especially helpful to farmers who are not present in the greenhouse during the day or who have other obligations and cannot find labor on weekends. The higher initial and continuing costs of mechanical ventilation systems are one of their drawbacks. This illustration shows how automatic control of greenhouse ventilation panes is possible.

Fan ventilation systems with well-thought-out inlets offer effective temperature control in all settings. The capability to seamlessly automate the entire system is its most alluring feature. This is now a reality thanks to the development of computer-based control systems. Farmers who have other responsibilities, lack workers on the weekends, or are away from the greenhouse during the day will find this function especially helpful. The disadvantages of mechanical ventilation systems are their high installation and maintenance costs. This illustration shows how automatic control of greenhouse ventilation panes is possible.

The greenhouse's owner wishes to have ventilation window panes on the roof of the greenhouse installed so that they can be opened and closed. There are two windows in the greenhouse for ventilation. Two sensors that detect whether the motor and the window panes are open or closed regulate how far these window panes open. The window panes open during the day to ventilate the building, ideally during the peak temperature of 12:00 to 15:00. However, window panes that are already open will not close or open if the temperature drops below 10 C. Additionally, on days when the temperature rises to 25 C, the windowpanes open.The window panes should be closed once more if the temperature drops below 25 degrees Celsius. No matter the temperature, window panes are closed at night. When the window panes are open, a red pilot lamp signal is displayed; when they are closed, a green pilot lamp signal is displayed.

Program description, 3-time ranges are used:

- Range 1: Night, from 21:00 to 07:00
- Range 2: Day, from 07:00 to 12:00 and from 15:00 to 21:00
  [T > 25° C – Opening , T < 25°C – Closing]
- Range 3: Noon, from 12:00 to 15:00
  [T > 10° C – Opening , T < 10°C – Closing]

**Benefits of an Industrial Automation Management system**

Automation of factories and industrial processes is made possible by industrial automation management systems, which enable continuous mass production seven days a week, 24 hours a day, increasing productivity and cutting down on assembly times. These systems help reduce human error and enhance the quality and uniformity of the products by employing adaptive control and monitoring throughout various industrial processes and stages. After working nonstop for several hours, the performance does not decline. Computers and other machines operate continuously and at a steady pace. As a result, when managed by an automation system, automated production processes have a longer lifespan, stability, and solidity. In a traditional production chain, implementing a new task

requires user training that can take hours or even days. However, reprogramming a robot or machine through an automated system is a quick and easy process that offers increased production process flexibility. Automation of data collection lowers expenses while increasing accuracy. Managers of the company are able to make better decisions because of the increased accuracy.

On manufacturing lines where human workers are exposed to hazardous conditions, using robots is a safer option. The Occupational Safety and Health Act was passed in the US in 1970 with the intention of enhancing worker protection and job safety. Since it was passed, it has encouraged the use of automation systems and robotics in the nation's factories. Industrial automation systems may require a large initial investment, but using this technology will result in lower costs for data analytics. Additionally, there is very little chance of a machine failure or service interruptions because of this automated data analysis. When an industrial automation management system is in place, workers in that factory put in less hours and spend more of it on high-value tasks. Employees are relieved of repetitive and laborious tasks by automation systems. Employees can perform more value-added work in other areas of the business that benefit the company more when machines and computers take over these tasks from them. In addition to carrying out tasks that a human would do, the systems that businesses use to automate their services are also capable of carrying out tasks that a human being could not accomplish. Among other things, they are superior in terms of size, weight, speed, and resistance. Some of the important strategies for best Industrial management practises is explained below.

**Forming the right team for Industrial Management**

Finding the right talent is one of the most important aspects of building a successful project team. Experts concur that a project management team does not need to be large, even though automation projects can be complicated. Veterans of the industry also contrast internalizing the automation project with working with a qualified System Integrator, citing the latter's high project success rate. Skilled system integrators "offer the value-added engineering services that concentrate on the unique requirements of your company." Put differently, they possess the necessary skills and resources to assist in creating a workable solution without having to start from scratch.

**Look for Technical Qualification in team member**

Inadequately qualified team members can have disastrous consequences for an automation project. Errors in project scoping, estimation, or assessment can become difficult and costly as the automation project moves forward. Businesses must ensure that all project participants have the necessary skills in PLC, HMI, hardware integration, software programming, network protocol, UI/UX design, and other related areas when developing solutions internally. Additionally, someone with the sole responsibility of choosing and evaluating vendors and suppliers is required. Instead, a lot of manufacturers choose to collaborate with System Integrators who already employ a group of skilled engineers. When a project team chooses to collaborate with outside parties, such as a system integrator, the members must be selected based on their ability to manage projects, think critically and analytically, and have experience with various production procedures.

**Appoint some one who understands Industry as a Project Manager**

Assign the project manager position to a knowledgeable industry professional. The project manager bears the responsibility of guiding the automation project towards its intended course. They are essential to an automation project because they serve as the point of contact for vendors and stakeholders. A qualified project manager's level of industry knowledge is one of the selection criteria. Gaining knowledge about the industry a company operates in, particularly in heavy industries, can take up to ten years. On the other hand, a well-informed project manager fosters team cohesion by being: Skilled in areas beyond their purview; knowledgeable about the offerings of rival companies; and able to make both internal and external connections.

**Partnership with system integrator wherever you can.**
System integrators are expert services that use commercially available hardware components to construct automation control systems and create software algorithms according to application requirements. By offering solutions and ongoing technical support, they assist manufacturers (end users) in automating jobs or processes.

**Knowing and applying current industry standards in safety, Environmental and modern technology.**
Skilled System Integrators collaborate with a diverse range of end users. They continuously monitor the most recent advancements in technology and industry best practices, including security, network standards, international programming standards, OSHA (Occupational safety and health Administrations) guidelines, machine safety, and process safety. It can be difficult to justify hiring an end-user for an automation project because they might not be skilled in every area.

**Choosing the best hardware and software for application**
It is the responsibility of a system integrator to be knowledgeable about various hardware components' interoperability. They are also capable of creating software applications and algorithms that interface with these parts. It takes years, if not decades, to become proficient at the complexity of creating an automation system. System engineers working for an organization often lack the time and resources necessary to become as proficient as an integration engineer whose entire career is focused on learning about system integration. Long-term returns on automation projects can be higher for end users who work with the right system integrator.

**Providing proper project documentation**
Future maintenance and upgrades to an automation system depend heavily on documentation. Usually, a system integrator oversees several installations at once. For future reference, it is in their best interest to maintain thorough documentation of the project. Regretfully, end users find it more difficult to obtain the same level of detail. Choosing the appropriate team members is the first step towards a successful automation project's execution. Members of the internal team should be technically proficient in both software programming and hardware technologies. A Project Manager with extensive industry knowledge and comprehension of various job functions is leading the team to success. Lastly, never undervalue the complexity of an industrial project. Reducing risks and expediting the project schedule can be achieved by collaborating with a qualified system integrator.

**Ensuring goals and roles are crystal clear**
Failure to communicate the project goals is akin to failing to inform a cab driver of your destination. It is a waste of money and time for both the driver and the passenger. The worst part is that it still falls short of the goal of the journey. Having well-defined objectives helps team members stay focused and minimize distractions, which enhances project success overall. An automation project will have numerous subtasks that must be completed at various stages of the project. Senior stakeholders and team members can communicate clearly when each member's specific responsibilities and milestones are defined. Devoid of well-defined responsibilities, team members who are driven and committed may experience animosity and exhaustion. Assigning responsibilities is undoubtedly crucial to fostering a team dynamic that functions like a well-oiled machine.

**Utilize project management technologies**
The preferred System Integrator may frequently reside in a different city. It is likely that the component suppliers it selects to work with are located in different states or nations. Even though they are convenient, sending emails or making phone calls can lead to misunderstandings and confusion. The integrity of the automation project is put at risk because it is difficult to maintain file version control in the absence of a centralized storage system. An increasing number of businesses are switching to device-synching project management software hosted on the cloud. These platforms can help with budget tracking, timeline management, data communication and storage, and—above all—documenting the project's progress. Project managers can better convey their vision and demonstrate leadership when they communicate well. A strong communication strategy facilitates the smooth adoption of the new solution by stakeholders, or potential system users. A team that communicates well will feel empowered and have a cohesive

group that can easily handle the numerous smaller tasks that make up an automation project. Ultimately, information synchronization and documentation between various parties and time zones can be facilitated by project management software.

**To spot scope creep and actions to be taken**

Scope creep is a dreaded phenomenon on any project that can result in wasted money and decreased satisfaction and a failure to meet the project's anticipated value. Scope creep appears to plague most projects, a problem that constantly irritates stakeholders and project teams alike. There are several ways in which scope creep can occur in projects. Executives at the sponsor level frequently state that they would prefer not to participate in every decision. Thus, they are created by project teams. Once more, because certain change requests appear to be minor, project teams handle them without adhering to a formal change management procedure. Strict or onerous change control procedures may also contribute to unapproved scope extensions. For a variety of reasons, the project team may feel compelled to go above and beyond and offer "more value" by adding functionality that hasn't been asked for. When requests for more functionality are made, IT managers frequently let the scope expand without pressing for more funding or time. Lack of scope definition, requirements and scope not managed, Inconsistent process for collecting requirements, lack of sponsorship or stake holder involvement and length of project are some key points to be considered.

**Lack of scope definition**

The project scope is stated in aspirational or lofty language. These include "better throughput" and "more cost-effective," which various stakeholders may interpret in different ways, leading to misunderstandings and confusion in the end.

**Unmanaged requirements and scope**

Emerging "discoveries" may change the requirements for a project. Scope creep happens gradually but steadily when these discoveries are added to the project without first undergoing a proper assessment.

**Inconsistent process for collecting requirements**

The cause of this phenomenon is imprecise scope definition, which leaves the project team unclear about the boundaries of the work. It can also occur when there are too many parties involved.

**Lack of sponsorship or stake holder involvement**

The project may lose momentum and focus if senior management or stakeholders are absent. The most frequent reason for project failures is a lack of sponsorship or stakeholder involvement.

**Length of project**

The likelihood of the project scope expanding exponentially increases as the timeline for the project gets longer. This occurs because senior management has more time to hone their concepts, research the competitors, or because the company evolves naturally over time.

## RESULTS AND DISCUSSION

This paper's primary goal is to present comprehensive data for plant automation, including ways to increase energy efficiency, match demand, increase utility, lower costs, and improve control and preserve Environment. By enhancing all of these aspects, the overall plant stability and utility is improved. How the effective temperature control and monitoring can be provided with Programmable logic controllers is explained very effectively. In boiler to prevent over consumption of electricity at the start of the boiler, When the boiler is turned off, the heating elements are gradually heated and turned off. The example of the greenhouse ventilation panels shows how automation can benefit the environment.

## CONCLUSION

The author has tried his best to justify the paper title Industrial plant automation Management best practises for great outcomes where not only the energy efficiency, emission, automation and programming is explained but the Industrial management aspects points are covered for best outcomes. Each automation project is distinct and intricate in its own right but few points are to be kept in mind. Establish specific goals and objectives and assess the project as a strategic business case using the company's mission, vision, and values. Choose the appropriate external partners and internal team members. Establishing proper communication mechanism with awareness of scope creep.

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**Table 1: Pros and cons associated with each control methods [7]**

| Control Method | Pros and cons |
|---|---|
| Logic for contacts and relays. | • Easy assembly and straight forward design.<br>• Simple to comprehend for small systems, requiring a lot of room and being rigid for large systems.<br>• Expensive regular,unkeep.<br>• More driver circuit are needed for large relays.<br>• Human involvement is inevitable.<br>• No intermediate control except tight on adjustment and on / off.<br>• Increases electrical noise and interference. |
| PLC apparatus | • Adaptable and practical for use.<br>• Boosts process dependability and efficiency.<br>• Has modest intelligence and cabling.<br>• Reusability and good fault tolerance. |
| SCADA setup | • Because of the system is scalable and modular, keep an eye on it all in real time.<br>• Easy to use interface with secondary messages and alarm for a particular element or plant area.<br>• Updating events and data logs for analysis and upcoming planning<br>• Extensive infrastructure Instrumentation sensors heavy, cabling etc.<br>• High Initial Outlay. |
| DCS setup | • Plug and play mostly in Easy implementation<br>• Measurable with modular and high reliability<br>• Low cabling and limited distribution intelligence<br>• Good wrong tolerance |

**Table 2: Thermostat status and temperature range**

| Temperature | Thermostats | Heater 1 | Heater 2 | Heater 3 |
|---|---|---|---|---|
| Lower than 55° C | TS1 Close<br>TS2 Close<br>TS3 Close<br>TS4 Close | ON | ON | ON |
| 55°C -60°C | TS1 Open<br>TS2 Close<br>TS3 Close<br>TS4 Close | OFF | ON | ON |
| 60°C -65°C | TS1 Open<br>TS2 Open<br>TS3 Close<br>TS4 Close | OFF | OFF | ON |
| 65°C-70°C | TS1 Open<br>TS2 Open<br>TS3 Open<br>TS4 Close | OFF | OFF | OFF |

| | | | | |
|---|---|---|---|---|
| Over 70 °C | TS1 Open<br>TS2 Open<br>TS3 Open<br>TS4 Open | OFF | OFF | OFF |

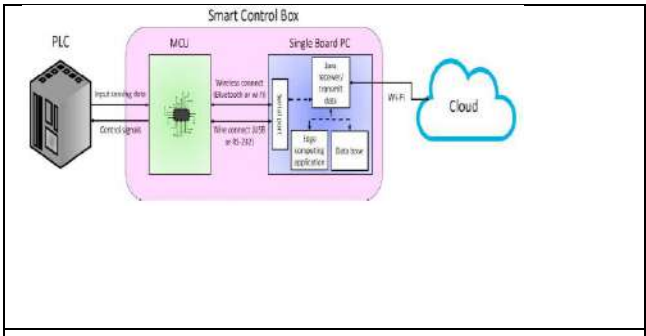


**Figure 1: Detail architecture of Smart Control Box [10]**

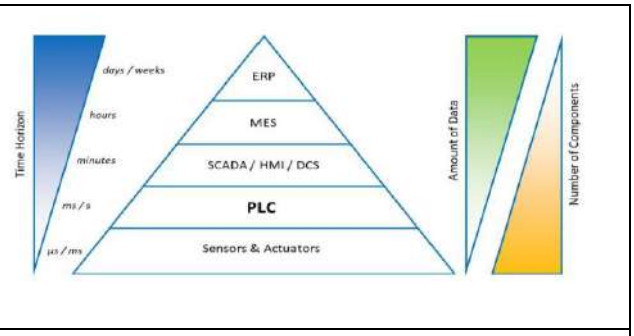


**Figure 2: PLCs form the control layer of the traditional automation pyramid**

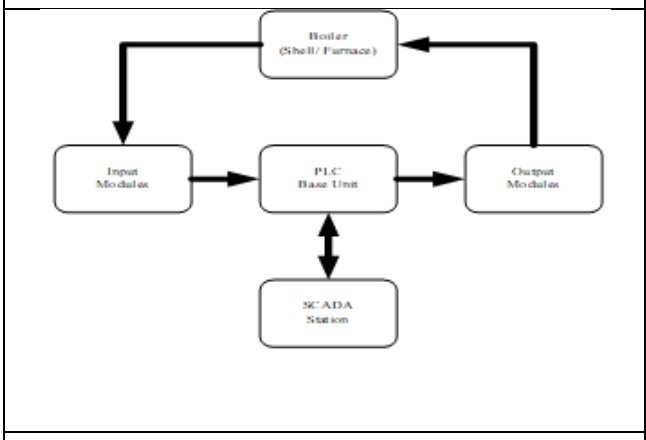


**Figure 3: General Interfacing of system [13].**

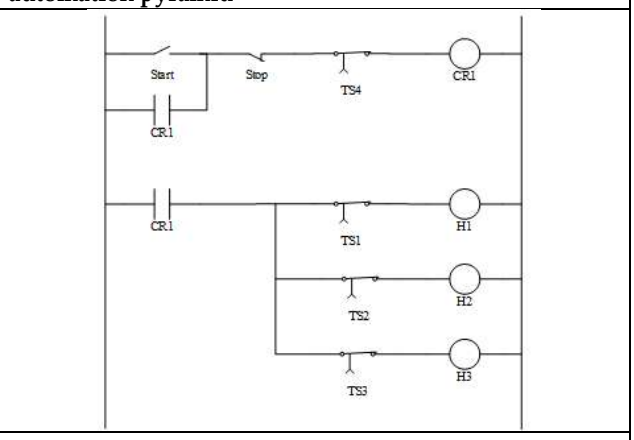


**Figure 4: Ladder Logic Operation**

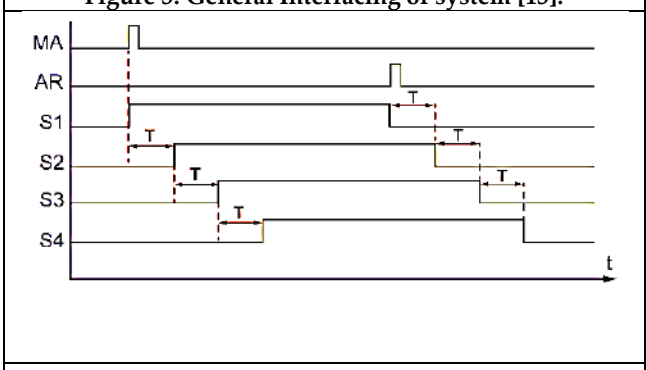


**Figure 5: Operating Principle of Boiler Control**

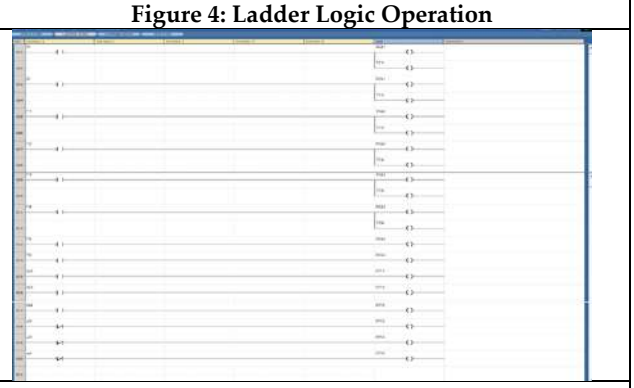

**Figure 6: Ladder logic program for the boiler control**

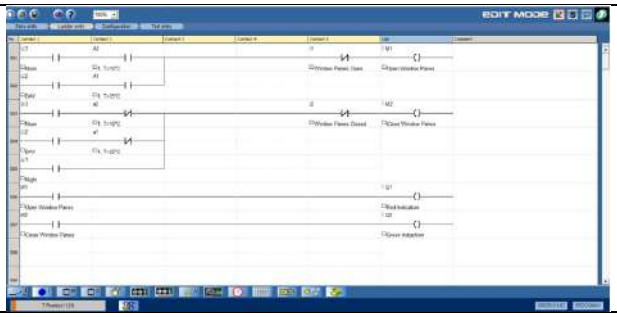


**Figure 7: Ladder Logic for Green House Ventilation pans**

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**RESEARCH ARTICLE**

# Management of *Karappan* (Eczema) with Siddha Formulation Sivappu Ennai as External Application – A Case Report

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**ABSTRACT**

Eczema is a form of dermatitis where inflammation of epidermis occurs. The exact cause of eczema is not known, although it is activated by the immune system and is related to allergic reactions. In Siddha, the disease is described by the name "*Karappan*" A 33-year-old male, complained of skin lesion over bilateral lower limbs associated with roughness, scaling and severe itching for 6 months. The case was diagnosed as *Karappan* (a Siddha diagnosis for Eczema) and *Sivappu ennai* was used to treat the patient for 6 months. Clinical symptoms and imaged software analysis of photographs of the lesions assessed the disease severity by EASI score. The treatment given was successful in reducing the symptoms. This case study demonstrates the effectiveness of *Sivappu ennai* on eczema patient. The study concluded that Siddha treatment was significantly effective in treating eczema.

**Keywords:** Eczema, *Karappan, Sivappu ennai*, Siddha.

## INTRODUCTION

The word eczema comes from the Greek word *ekzein* meaning "to boil out"; word *ek* means "out", while *zema* means boiling. (1) The exact cause of eczema is unknown. People with eczema do have the IgE antibodies (Immunoglobulin E) produced by the immune system as part of allergic reactions. Eczema can be difficult and frustrating condition. The psychological challenge faced by patients of eczema is insurmountable. The natural human desire to scratch an itchy rash just makes the condition worse. (2) In Siddha eczema is defined as *Karappan*(3)

**Case presentation**

A 33- year-old male presented with the complaint of rashes over bilateral lower limbs associated with intense itching for past 6 months was reported at Post graduate Noi Naadal outpatient department at Aringnar Anna Government Hospital of Indian Medicine, Arumbakkam, Chennai. He had oozing of fluid associated with burning sensation and dry scaly lesions over the popliteal fossa. There was tenderness over the lesions with elevated local temperature and surrounding indurations. He had disturbed sleep due to burning sensation and itching over both lower limbs. There was no relevant family history related to his illness. Further he had no past history of any major illness or surgery. He was psychologically disturbed due to stress and there was disturbed sleep.

**Clinical findings on examination**

Inspection of the skin lesions showed dark blackish discoloration with an irregular margin on the bilateral lower limbs. On palpation, the pulses of the lower limbs were normal. The skin lesions on the bilateral lower limbs showed macular papule lesions with irregular shapes and borders.

**Patient consent**

Written permission for the publication of the images has been obtained from the patient.

**Siddha Intervention**

He was given *Sivappu ennai* as external application and advised to apply over the affected areas morning and night daily till the skin color changes to normal. He was advised to visit weekly once to the OPD and advised to avoid brinjal, fish, crab, prawn foods. The EASI score assessment and the timeline of the treatment of the patient was given below in the table 1,2 respectively.

**Outcome and Follow up**

The assessment was done before treatment, on follow up visits and after the treatment based on differences in sign and symptoms. Image analysis helped to differentiate the disease and normal skin areas. EASI score was used to assess the improvement. Before treatment the patient had 16.8 EASI score and after treatment the EASI score was reduced into 0.8 as the skin color changed to normal. At the end of 45$^{th}$ day he recovered completely in all subjective and objective parameters. The patient was advised to follow up for next 2 months with continuation of the same medicines and there was no recurrence of the symptoms noticed.

## DISCUSSION

"Itch that rashes" is characteristic feature of Eczema.[4] The lipid barrier of skin is usually reduced in the people with eczema, compared with others. The lipid barrier helps prevent water loss. As the barrier is reduced water loss will be faster as a result skin becomes dry. The immune system then overreacts to these allergens and causes inflamed, irritated, or sore skin. Before treatment, the patient had hyperpigmented patches over bilateral lower limbs along with oozing of fluid and burning sensation over the affected area (Figure1). After a course of 45 days administration of both internal and external Siddha medicines, the patient was completely relieved from itching, oozing of fluid, burning sensation. Notable changes had occurred over the hyperpigmented patches in the bilateral lower limbs. (Figure2). Along with internal and external medication Pathiyam (diet protocol suitable to the patient and disease) was advised according to Siddha system of medicine in the management of eczema. Hence, the patient was advised to avoid bitter gourd, brinjal, tamarind, chicken, egg, sea foods, fast food, and baked items. Considering etiology, the patient had a history of consumption of sweet, cold, and colored food items from the bakery and he did not drink sufficient water, which resulted in the lipid barrier damage to the skin that leads to itching, blackish discoloration of the bilateral lower limbs, exfoliation, and skin roughness.

**Patient Perspective**

Patient was satisfied with the treatment in terms of reduced itching, burning sensation and improved sleep.

## CONCLUSION

Siddha management of eczema offers a comprehensive, individualized approach that addresses the root causes and manifestations of the disease. A remarkable change in the symptoms was noticed and provided a better outcome. Following strict dietary protocol along with both internal and external medication followed by proper lifestyle changes are the essential to prevent recurrences and sustained relief after the treatment.

**Limitations**

As this is a case study, the results cannot be generalized. In future, long-term follow-up studies on large samples are required to substantiate the above claims.

## ACKNOWLEDGEMENTS

I acknowledge the patient and his family for giving their consent for the images taken and publication of the same.

**Author's Contributions**

All the authors contributed equally to the design and execution of the article.

**Conflicts of interest**

Nil

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**Table 1: Eczema Area and Severity Index (EASI Score)**

| Score | Description |
|---|---|
| 0 | Clear or no lesions |
| 0.1-1 | Almost clear |
| 1.1-7 | Mild disease |
| 7.1-21 | Moderate disease |
| 21.1-50 | Severe disease |
| >51 | Very severe disease |

**Table 2: Timeline of Symptoms and Treatment with EASI score Assesment**

| Days | Symptoms | Internal& External Medicine | EASI Score |
|---|---|---|---|
| 1-10 | Oozing of fluid slightly reduced, Itching, burning sensation and hyperpigmentation present. | *Parangipattai chooranam*- 2gm BD with warm milk, after food | 16.8 |

| | | | |
|---|---|---|---|
| 10-20 | Oozing of fluid completely reduced. burning sensation decreased and itching slightly reduced. | *Sangu parapam*-100mg BD with warm milk, after food | 12.8 |
| 20-30 | Burning sensation completely relieved, hyperpigmentation reduced with mild itching. | *Silasathu parpam*- 100mg BD with warm milk, after food | 6.8 |
| 30-45 | Itching completely reduced, Skin color changed to normal and patient felt normal. | *Sivappu ennai* as external application twice daily(day&night) | 0.8 |

| | |
|---|---|
| | |
| Figure 1 | Figure 2 |

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**RESEARCH ARTICLE**

# Assessment of Developed Temperature Tolerant Yeast Strains for Fermentation Efficiency

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## ABSTRACT

Fuel substituted for gasoline primarily consists of bioethanol. Because of the high cost of crude oil, the need of biofuel increased. The primary method to generate bioethanol fuel is the fermentation of sugar, while it may also be produced using a variety of chemical processes, such as combining ethylene with steam. Using ethanol as a biofuel or as a blended fuel can help to reduce air pollution, carbon dioxide buildup, global climate change, and reliance on foreign energy. Industries nowadays use variety of raw materials for the production of ethanol such as sugar cane molasses, beet molasses, sweet sorghum and grains etc. *Saccharomyces cerevisiae* is the most commonly used microbe used to generate bioethanol from sugar-containing feed stocks because of its ability to break down sucrose into hexoses, which include glucose and fructose. *Saccharomyces cerevisiae* functions under aerobic and anaerobic conditions. The primary product of anaerobic yeast sugar fermentation is ethanol. Following the conversion of di-saccharides like sucrose in molasses into mono-saccharides like glucose and fructose by the yeast enzyme "Invertase," the yeast enzyme "Zymase" thereafter converts the mono-saccharides into ethyl alcohol and carbon dioxide. The production of ethanol by *S.cerevisiae* is affected by numerous factors which include culture medium, dissolved oxygen, immobilisation, and other micronutrients, as well as fermentation conditions such as temperature, pH, ethanol concentration, and sugar concentration. At high temperature, growth and viability of yeast cells reduces and hence the fermentative capacity and ethanol yield. In this study, temperature tolerant yeast strains were developed using Molasses Medium and Yeast Extract Peptone Dextrose Medium on applying heat stress. Based on the usual characteristics of colonies grown on Yeast Extract Peptone Dextrose Agar, the isolates were picked up for their morphological and physiological characterization. The developed temperature tolerant strains were evaluated for their fermentation capacity to ferment sugars and produce ethanol. . The results revealed that all the developed temperature tolerant strains were able to grow well at temperatures- 20°C, 25°C, 30°C, 35°C, 40°C and 45°C.

Conventional morphological and biochemical methods were used to characterize the developed strains and were compared with the wild commercial yeast cultures. The results reported that all of the developed temperature tolerant strains are of *Saccharomyces cerevisiae.* All the developed temperature tolerant yeast strains were able to produce ethanol in an anaerobic condition to a different extent due to the difference in temperature. The fermentation efficiency of temperature tolerant strains at 20°C and 25°C was ranging between 90 to 93%. The fermentation efficiency of temperature tolerant strains at 40°C and 45°C was ranging between 81 to 92%. The temperature tolerant strains 35M (35°C) – molasses medium based culture and 35Y (35°C) – YPD Broth Medium based cultures achieved the maximum fermentation efficiency of 99.6% and 99.6% respectively. The temperature tolerant strains developed could be used in distilleries that are located in the region having different climatic conditions with temperature ranging between 20-45°C accordingly for better fermentation efficiency.

**Keywords:** Distillery; Ethanol; Molasses; YPD medium, Yeast and Temperature Tolerance.

## INTRODUCTION

Microorganisms such as bacteria, yeast, fungi, protozoa, and viruses have substantial effects on the environment. These microbes possess positive as well as negative impacts on the areas such as Agriculture, Food Industry, Distillery, Health sector etc. Regarding the distillery sector, the energy crisis forces research and development of novel techniques for the synthesis of renewable compounds as substitute energy sources; one important substitute is the fermentation of ethanol using renewable resources. Fuel that is environmentally benign and suitable for use in stock gasoline engines is bioethanol. Ethanol combustion produces comparatively little emissions of carbon monoxide, nitrogen oxides, and volatile organic compounds. Ethanol has less toxicity and emissions than fossil fuels like diesel, petroleum, and so on. Global ethanol production increased from 13.12 billions of gallons in 2007 to 25.68 billions of gallons in 2015 with a slight decreased in 2012 and 2013[1].Theuse of bioethanol instead of gasoline has a variety of benefits, including a higher octane number (108), wider flammability limits, higher flame speed, greater heat of vaporization, less toxicity, easier biodegradation, and a lower amount of airborne pollution [2].Yeasts are those microbes which are solely responsible for the process of production of ethanol. Many types of yeast strains have been identified all over the world with the ability of producing ethanol from different types of feedstocks. 'Wild' yeast strains, or the yeast population that exists naturally on the fermentation feedstock or other components, have been used to produce alcohol (ethanol).Traditionally, baker's yeast was utilized as a beginning culture in the synthesis of ethanol since it was inexpensive and readily available. However, during industrial procedures, contamination occurred because baker's yeast and other *S. cerevisiae* strains were unable to compete with wild-type yeast [3].

Yeasts like *Saccharomyces cerevisiae* have been employed to produce alcohol for thousands of years, mostly in the wine and brewing sectors. Approximately 80% of ethanol is produced by anaerobic fermentation of various sugar sources by *Saccharomyces cerevisiae* [4]. It produces a high ethanol output, is very productive, and can tolerate high ethanol concentration; it keeps the cost of distillation low [5]. *S. cerevisiae* which is commonly called as fermentative yeast, used in ethanol production largely depends upon renewable biomass such as sugar cane or sugar beet molasses as the main carbon source [6, 7]. Yeast requires building block components (C, N, P, S, and O) in the right proportions, minerals (K, Na, Mg, Ca, Zn, Fe, Mn, Cu, and Co), and vitamins (B1, B5, B6, Biotin, etc.) for efficient fermentation. In commercial-scale ethanol fermentations, oxygen is often present at very low concentrations. Since oxygen is needed to produce unsaturated fatty acids, which are necessary for yeast growth and ethanol synthesis, the process cannot actually be entirely anaerobic. It has been discovered that the majority of substrates used in commercial ethanol production are nitrogen constrained [8].Various strains of yeast, including hybrid, recombinant, and wild-type yeasts, for the production of bioethanol, a range of feedstocks from the first, second, and third generations have been used [9]. Feedstocks high in starch (corn, wheat, rice, potato, cassava, sweet potato, and barley) and sucrose (sugar cane, sugar beet, sweet sorghum, and fruits) are used to produce first-generation bioethanol. Bioethanol of the second generation is derived from lingo cellulosic biomass, which includes grasses, wood, and straw. Micro and macroalgal biomass has

been used to produce third-generation bioethanol [10]. The fermentation process that results from using molasses to produce ethanol will add value; to the byproduct. The non-crystalllizable residue that remains after purifying sucrose is called molasses. It offers a few benefits, including being a commonly available, reasonably priced raw material that doesn't require starch hydrolysis and has already been used to produce ethanol. After sugar beets are processed, molasses is produced that comprises 40% additional ingredients and roughly 60% sucrose. The non-sucrose substances consist of nitrogen-containing molecules, organic acids, raffinose, ketose, and inorganic salts. Molasses is utilized to generate acetone, butanol, glycerol, citric, lactic, and gluconic acids; it is also employed to make baker's yeast, ethanol, mixed feeds, and amino acid manufacturing been employed in fermentation to produce ethanol. While other types of feedstocks need to first be converted into fermentable sugars in order so as to be fermented to ethanol, simple sugars can be directly fermented into ethanol by yeasts [11, 12]. In India, cane juice is not currently used to produce ethanol; instead, sugar cane molasses is the primary feedstock. Another source of fermentable sugars for the production of ethanol is beet molasses [13].

Strains, growth nutrients, ethanol concentration and ideal climatic conditions are only a few of the variables that affect yeast's capacity to produce ethanol. Yeast's ability to tolerate its environment, including temperature, fermentation products, and substrate (osmo- and thermo-tolerance), has significant potential to be utilized in fermentation on an industrial scale[14].The ecology and adaptation of the existing microbiota can be influenced by several factors that impact the fermentation process. During the fermentation of sugar, yeasts are frequently faced with two challenges: an increase in temperature from 35 to 45°C and a concentration of ethanol exceeding 20%. Until the temperature reaches its ideal level, the growth rate and metabolism of yeast increase with temperature [15].One factor that directly influences the rate at which the microbes grow is the temperature.A temperature shift of a few degrees to twenty degrees Celsius over the usual growth temperature is referred to as heat shock. There is a general relationship between the rate of growth and the susceptibility to stress in yeast, meaning that cells developing rapidly in a glucose-rich media are more susceptible to heat and other stresses than stationary phase cells [16].Heat shock causes changes in the lipid and protein content of membranes as well as the physical states of intracellular water, which increases the permeability of membranes. Saturated esterified fatty acids, such palmitic and palmitoleic acids, are more abundant in yeast cell membranes at the expense of unsaturated acyl chains, like oleic, linoleic, and linolenic acid, as temperature rises. This is typically linked to a reduction in the level of phospholipids in the membrane necessary to maintain ideal membrane fluidity for cellular processes, some of which may be adaptive in nature. The heat shock response, the cell's survival mechanism in the midst of a stressful environmental situation, is triggered by these biochemical changes and the accumulation of unusual proteins in the cell [17]. *Saccharomyces cerevisiae* is used in industrial fermentations, which produces metabolic heat. If cooling is not applied, the fermentation will stop being isothermic. From the start of fermentation to its conclusion, temperatures might rise as much as 15°C [13].With the goal to achieve maximum ethanol production while reducing energy costs, thermo-tolerant yeast is required.Many species of yeast, including *Pichia* sp., *Candida* sp., *Kluyveromyces marxianus*, and certain strains of *Saccharomyces cerevisiae*, have been identified and categorized as thermo-tolerant yeasts. The ideal temperature range for the majority of *Saccharomyces cerevisiae* strains is between 25°C and 30°C. Using chemical and physical mutagenesis techniques, some thermo-tolerant mutants of haploid strains of *S. cerevisiae* have been identified and genetically described [18].

## MATERIALS AND METHODS

**Collection and Primary Analysis of the Sample**

B Heavy Molasses sample was obtained from Experimental Sugar Factory, National Sugar Institute, Kanpur. Analysis of the molasses sample was done based on the parameters such as Total Reducing Sugar (TRS), Reducing Sugar (RS), Brix, Specific Gravity, Sludge, Total Dissolved Solids, Alcohol Percentage and pH. The molasses sample was diluted to 10% TRS, analyzed based on the above parameters.

**Fermentation**

The diluted molasses sample was supplemented with Commercial Yeast, Urea and Sodium Phosphate Dibasic Anhydrous and kept for aerobic and anaerobic fermentation at 30°C for 24 hours

**Growth on Medium**

A pre-grown yeast culture from aerobic flask was inoculated into molasses medium and YPD Broth Medium (Yeast Extract-10g, Peptone- 20g, Dextrose- 2g, and Distilled Water-1000 ml). pH of both the medium was adjusted between 4.5-5.0 **[19].** Both the mediums were incubated at 6 different temperatures- 20°C, 25°C, 30°C, 35°C, 40°C and 45°C for 24 hours. Cell viability was determined for each of the twelve samples.

**Isolation, Identification and Characterization**

Yeast cultures from Molasses and YPD Broth Medium was streaked on YPDA and incubated at 6 different temperatures for 24-48 hours. Pure culture was obtained for each isolate. Morphological characters were studied using macroscopic and microscopic features. Macroscopic characters such as shape colour, elevation, texture of the isolated colony was studied and Microscopic traits such as shape of the yeast isolate, cell arrangement and budding was observed. Biochemical characterization was done by Carbohydrate Fermentation Test using Durham Tube Method.Durham tubes were utilized to test yeasts for the fermentation of carbohydrates. YP Broth was used for characterization of the yeast isolates based on fermentation of specific carbohydrates. The sugars used for the test were dextrose, sucrose, fructose, trehalose, starch, $\alpha$-methylglucoside, galactose, maltose and mannitol. The basal media for the fermentation test includes Yeast Extract- 1g, Peptone- 2g and Sugar- 2g, Distilled Water-100ml and 0.1% Methyl Red Indicator. Durham Tube was used to observe the formation of gas bubbles and Methyl Red Indicator was used for observing the color change of the media; from red to yellow due to production of acids [20]. Pellicle formation was also observed for each yeast isolate [21].

**Distillation and Fermentation Efficiency**

Yeast cultures from YPDA slants of Molasses and YPD Broth Medium was transferred to molasses medium for aerobic fermentation at 6 different temperatures for 24 hours. After 24 hours cultures from aerobic flask of Molasses and YPD Broth Medium was transferred into molasses medium (100ml each) for anaerobic fermentation at 6 different temperatures for 24 hours. After fermentation distillation was performed for each yeast isolate, strength of the alcohol was determined and fermentation efficiency.

## RESULT AND DISCUSSION

**Primary Analysis of the Molasses**

B Heavy Molasses sample for the study was obtained from the Experimental Factory of National Sugar Institute, Kanpur and was analyzed for various parameters. The result of the analysis carried out is given in the Table 1. The Total Reducing Sugar, Reducing Sugar, Specific Gravity, Sludge, Brix, pH and Total Dissolved Solid of the B Heavy Molasses sample utilized for the study was calculated as 54.1%, 2.4%, 1.42, 11.4%, 84°, 4.8 and 35.0 PPT respectively. The molasses sample obtained from NSI was diluted to 10% for further study.

**Aerobic and Anaerobic Fermentation**

For the purpose of aerobic and anaerobic fermentation the molasses having the TRS 54.1% was diluted as per the formula N1V1= N2V2 to 10% and various parameters was analyzed for the same. The Total Reducing Sugar, Reducing Sugar, Specific Gravity, Sludge, Brix, pH, Total Dissolved Solid and Alcohol Percentage of the diluted Molasses sample was calculated as 9.8%, 0.02%, 1.42, 8.3%, 28°, 4.8, 3.5 PPT and 5.6% respectively. The molasses sample was subjected to aerobic and anaerobic fermentation. Cell counting of molasses medium from aerobic fermentation was carried out using Hemocytometer (Table 2) and molasses medium for anaerobic fermentation was subjected to distillation to determine the strength of alcohol.

Following aerobic fermentation for 24 Hrs at 30°C, Total Cell Count was calculated as $315\times10^6$Cells/ml, Dead Cells were found to be 1.58%, Viable Cells and Bud Count was found to be 98.4% and 15.8 % respectively.

**Growing of Yeast on Molasses Medium and YPD Broth Medium**

Both Molasses and YPD Broth Medium were prepared and taken in different flasks marked as 20M (20°C), 25M (25°C), 30M (30°C), 35M (35°C), 40M (40°C) and 45M (45°C) and 20Y (20°C), 25Y (25°C), 30Y (30°C), 35Y (35°C), 40Y (40°C) and 45Y (45°C). Both Molasses and YPD Broth Medium were kept at respective temperatures (20°C, 25°C, 30°C, 35°C, 40°C and 45°C). This is followed by inoculation of yeast culture from aerobically fermented culture. The growth of yeast in both Molasses and YPD Broth Medium was observed after 24hrs of incubation. The growth of yeast cultures was found to be increasing with temperature from 20°C to 35°C and the total cell count decreased at 40°Cand till 45°C incubation. This pattern of growth was observed in both Molasses and YPD Broth Medium. The four strains namely VS1, VS2, VS3 and VS4 were able to grow well in broth cultures till 44°C [22].

**Determination of Cell Viability**

Hemocytometer was used to assess the vitality of the cells in all the inoculated flasks after 24-hrs. The total number of cells, viable cells, dead cells and budding count was calculated andthe values are given in the Figure 2 and 3 for cultures grown in Molasses and YPD medium at different temperatures. The cell counting was found to be highest in the case of 35M and 35Y (grown at 35°C); it was found that the total cell count first increased as the temperature increases up to 35°C then it decreased until it reaches 45°C. The dead cells were not found in case of 25M (25°C), 30°C (30M and 30Y) and 35°C (35M and 35Y) whereas with increasing the temperature the dead cells. Were also increased due to the stress created by the temperature. The viability of the cells first increased up to 35°C with increase in temperature and then decreased. The maximum percentage of bud count was found to be maximum at 35°C (35M and 35Y).

**Isolation of Temperature Tolerant Yeast Strains on YPDA Medium**

The yeast cultures grown on Molasses and YPD Broth Medium at different temperatures were isolated on YPDA Medium by streak plate method. Isolated yeast from both the medium grown at different temperatures is shown in the Figure 4 and 5. The yeast isolates from both the medium grew well at 30°C and 35°C. The growth of the yeast isolates decreased as the temperature rise above 35°C. Out of four strains used (VS1, VS2, VS3 and VS4), VS1 and VS3 grew well on plates till 44°C but the growth of VS2 and VS4 was very less at 44°C [22]. Four strains namely LGBA-01, LGBA-69, LGBA-157 and LGBA-175 and all the strains had same pattern of growth at 30°C and 40°C [23]. In the present study, the isolated yeast colonieswere found to be white in colour, smooth in texture, margin entire.Yeast colonies from different food waste were found to be smooth ridged, shiny and creamish[24]. Colony colorof yeast strains from palm wine were ranging from cream white to colorless [25].

**Pure Culture of the Yeast Isolates**

Pure Culture was prepared by picking single isolated colonies obtained on petriplates from Molasses Medium Based Cultures and YPD Broth Medium Based Cultures and streaking it on YPDA slants (shown in Figure 6 and 7).The growth of pure culture of Yeast Culture was observed after 72hrs of incubation at 20°C, 25°C, 30°C, 35°C, 40°C and 45°C. The yeast isolates from Molasses Medium and YPD Broth Medium on petriplates were examined for colour, texture, margin, shape, elevation and colony size as indicated in Table 3 and Table 4. All of the Yeast Cultures isolated from Molasses Medium Based Cultures were white in colour. The texture was observed as smooth, with an entire margin. The shape was found to be circular with a convex elevation. The colony size of 30M and 35M isolates were found to be 2×2 mm while 25M was significantly larger, measuring 3×2.5 mm. In the case of Yeast Cultures isolated from YPD Broth Medium the colonies were white in colour, smooth in texture, with an entire margin, circular in shape, and convex in elevation. The colony size of 45Y was found to be highest as 3×2.5 mm. Yeast cultures isolated, from soil samples and fruit gardens showed a rough surface with a white or creamy white tint, the majority of colonies had a smooth surface. Most of the colonies margins were lobate, serrated, or entire [26]. On macroscopic observations of yeast isolates from palm wine, the elevation of all the isolates was found to be raised. Hence they

reported that the colonies of yeast strains possessed morphological features which corresponded to *S. cerevisiae* species [25].

**Microscopic Characteristics**

Lactophenol Cotton Blue Staining was done to confirm the shape of the yeast cell and the formation of buds under the microscope (Table 5 and 6).The cell shape of the yeast cultures from molasses medium based cultureswas found to be oval. The shape was round in the case of 30M (30°C) and 45M (45°C). The cell arrangement was isolated, and some of them were grouped in tiny clusters. Budding was observed in all the cultures. In the case of yeast cultures from YPD Broth Medium Based Cultures the cell shape was oval and round. The cell arrangement was isolated, with some forming small clusters. Budding was observed in all the cultures. The isolated yeast cultures were ovoid in shape and multipolar budding was observed [19]. Yeast from palm wine were found to have an ovoid to circular shape, the cells appeared singly and varied in size from 2 to 6 μm[25]. The yeast isolates had a range of cell morphologies, including spherical, ovoid, elongated elliptical, and cylinder, with lengths between 3 and 11 μm and widths between 2 and 6 μm[26].

**Pellicle Formation**

Pellicle Formation was examined by inoculating the yeast cultures in YPD Broth Medium followed by incubation for 48 hrs. The results are shown in Figure 8 and 9. Pellicle formation was not observed in any of the cultures of yeast cultures isolated from molasses medium based cultures as well as YPD broth medium based cultures. Yeast cultures from samples of soil, fruit, and fermented food were isolated none of them formed pellicle **[21]**.

**Biochemical Test**

The Carbohydrate Fermentation Test was used to determine the ability of the yeast isolate to ferment specific sugar by releasing carbon dioxide and changing the colour of the medium. It was done by inoculating cultures of yeast in basal medium with Durham's tube and the indicator methyl red. It was done using nine different carbohydrate sources which were numbered as Dextrose-1, Starch-2, Fructose-3, Trehalose-4, Maltose-5, $\alpha$-Methylglucoside-6, Mannitol-7, Sucrose-8 and Galactose-9. The results are shown in Table 7 and Figure 10.

All the yeast cultures from molasses medium based cultures as well as YPD broth medium based cultures were able to ferment following 72hrs of incubation. The isolates were observed to be fermenting reducing sugars (Dextrose, Fructose, Galactose, Trehalose, Mannitol and Maltose) and non reducing sugar (Sucrose and $\alpha$-Methylglucoside).The yeast cultures were unable to ferment starch even after 72hrs.Out of 20 thermotolerant strains only 4 of them (YP11, YM17, YPA48, YPA64) were able to ferment all the nine sugars used (Glucose, Galactose, Sucrose, Mannose, Lactose, Raffinose, Arabinose and Xylose and Fructose) [24]. The yeast isolated from wine samples were subjected to biochemical assimilation test using various sugars like Glucose, Sucrose, Maltose, Xylose, Galactose, Lactose,Raffinose, Melibiose, Mannitol, Trehalose, Erythritol. The ability of yeast to assimilate Glucose,Raffinose and Maltose confirmed for *S. cerevisiae* [25].

**Fermentation Efficiency of Temperature Tolerant Strains**

The Yeast Cultures were kept for anaerobic fermentation followed by distillation. The fermentation efficiency of the temperature tolerant yeast strains was calculated. The results of the analysis are shown in Figure 11. In the case of Molasses Medium Based Cultures, the isolate 35M (grown at 35°C) was found to have the highest Practical Yield and Fermentation Efficiency (5.15 and 99.6% respectively). In the case of YPD Broth Medium Based Cultures, the isolate 35Y (grown at 35°C) was found to have the highest Practical Yield and Fermentation Efficiency (5.20 and 99.6% respectively). According to [27] the strain K-7 yields 6.2% ethanol at 40°C after 48 hrs of fermentation with total sugar concentration of 23% and the strain RND 13 produced 6.6% ethanol at 40°C at 15% glucose concentration. Whereas in the present study, at 40°C the strain 40M and 40Y yields 2.61% and 2.65% ethanol respectively at 10% TRS.

The concentrations of ethanol were lowest at higher temperatures and maximum at 30°C and 35°C. Even though the cells were producing less ethanol and growing at 44°C, they were still viable at higher temperatures. The four strains

VS1, VS2, VS3 and VS4 produced ethanol (g/l) - 66.0, 50.0, 75.2 and 55.0 respectively at 30°C using 150g glucose/l. All these strain produced ethanol (g/l) – 66.0, 48.0, 75.0 and 52.0 respectively at 35°Cusing 150g glucose/l. All the above data were reported by [22]. Where as in the present study, at 30°C the strain 30M and 30Y yields 5.0% and 5.18% ethanol respectively at 10% TRS. At 35°C the strain 35M and 35Y yields 5.15% and 5.20% ethanol respectively at 10% TRS.

## CONCLUSION

The experimental results reported in the present study revealed that twelve temperature tolerant yeast strains were developed viz Molasses Medium- 20M, 25M, 30M, 35M, 40M and 45M and YPD Broth Medium-20Y, 25Y, 30Y, 35Y, 40Y and 45Y. All the developed temperature tolerant strains were able to grow at their respective temperatures (20°C, 25°C, 30°C, 35°C, 40°C and 45°C). By comparing the developed temperature-tolerant strains with wild commercial yeast cultures, standard morphological (macroscopic and microscopic) analysis and biochemical approaches were used to characterize them. Upon performing the tests, the developed strains were confirmed to be *Saccharomyces cerevisiae.* All the developed temperature tolerant yeast strains were able to produce ethanol in an anaerobic condition to a different extent due to the difference in temperature. In order to assess the ability of the strain to produce ethanol, fermentation efficiency was calculated for all the twelve temperature tolerant yeast strains. The results showed that the temperature tolerant strain 35M and 35Y had the maximum efficiency of 99.6% and 99.6%. Though the optimum temperature of *Saccharomyces cerevisiae* to grow is 25°C to 35°C; the results of the present study showed that at 20°C, the fermentation efficiency was found to be 90.3% and 91.7% which means the developed temperature tolerant strains was able to ferment sugar and produce 3.57% and 3.34% at 20°C. In order to improve fermentation efficiency, temperature-tolerant strains that have been produced could be employed in distilleries situated in regions with varying climatic conditions, with temperatures ranging from 20 to 45°C.

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### CONTRIBUTIONS

Ananthalakshmi , Shubhi Bhagwat and Anjali Yadav were involved in research and analysis. Seema Paroha and Sonali Nigam helped in reviewing the paper.

### CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

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**Table 1: Analysis of B Heavy Molasses Before and After Dilution**

| Parameters | B Heavy Molasses | Diluted Molasses (10%) |
|---|---|---|
| TRS (%) | 54.1 ± 0.1 | 9.8 ± 0.1 |
| RS (%) | 2.4 ± 0.1 | 0.02 ± 0.01 |
| Specific Gravity | 1.42 ± 0.01 | 1.050 ± 0.01 |
| Sludge (%) | 11.4 ± 0.1 | 8.3 ± 0.1 |
| Brix (°) | 84 ± 1 | 28 ± 1 |
| pH | 4.8 ± 0.1 | 4.8 ± 0.1 |
| TDS (PPT) | 35.0 ± 0.1 | 3.5 ± 0.1 |
| Alcohol (%) | - | 5.6 ± 0.2 |

**Table 2: Cell Counting of Molasses Medium from Aerobic Fermentation**

| Cell Counting | |
|---|---|
| Total Cell Count (Cells/ml) | $315\times10^6$ |
| Dead Cells (%) | 1.58 |
| Viable Cells (%) | 98.4 |
| Bud Count (%) | 15.8 |

**Table 3: Macroscopic characteristics of yeast isolate from Molasses Medium Based Cultures**

| Sample | Colour | Shape | Texture | Margin | Elevation | Colony Size (mm) |
|---|---|---|---|---|---|---|
| 20M | White | Circular | Smooth | Entire | Convex | 2×2.5 |
| 25M | White | Circular | Smooth | Entire | Convex | 3×2.5 |
| 30M | White | Circular | Smooth | Entire | Convex | 2×2 |
| 35M | White | Circular | Smooth | Entire | Convex | 2×2 |
| 40M | White | Circular | Smooth | Entire | Convex | 1.5×1.5 |
| 45M | White | Circular | Smooth | Entire | Convex | 2×2.5 |

**Table 4: Macroscopic characteristics of yeast isolates from YPD Broth Medium Based Cultures**

| Sample | Colour | Shape | Texture | Margin | Elevation | Colony Size (mm) |
|---|---|---|---|---|---|---|
| 20Y | White | Circular | Smooth | Entire | Convex | 2×2 |
| 25Y | White | Circular | Smooth | Entire | Convex | 1.5×2 |
| 30Y | White | Circular | Smooth | Entire | Convex | 2×2.5 |
| 35Y | White | Circular | Smooth | Entire | Convex | 1.5×2.5 |
| 40Y | White | Circular | Smooth | Entire | Convex | 2×2 |
| 45Y | White | Circular | Smooth | Entire | Convex | 3×2.5 |

**Table 5: Microscopic characteristics of Yeast Cultures isolated from Molasses Medium Based Cultures**

| Sample | Shape | Cell Arrangement | Budding |
|---|---|---|---|
| 20Y | Oval | Isolated or grouped in small clusters | Present |
| 25Y | Oval and Round | Isolated or grouped in small clusters | Present |
| 30Y | Oval | Isolated or grouped in small clusters | Present |
| 35Y | Oval and Round | Isolated or grouped in small clusters | Present |
| 40Y | Oval | Isolated or grouped in small clusters | Present |
| 45Y | Oval and Round | Isolated or grouped in small clusters | Present |

**Table 6: Microscopic characteristics of Yeast Cultures isolated from YPD Broth Medium**

| Sample | Shape | Cell Arrangement | Budding |
|---|---|---|---|
| 20Y | Oval | Isolated or grouped in small clusters | Present |
| 25Y | Oval and Round | Isolated or grouped in small clusters | Present |
| 30Y | Oval | Isolated or grouped in small clusters | Present |
| 35Y | Oval and Round | Isolated or grouped in small clusters | Present |
| 40Y | Oval | Isolated or grouped in small clusters | Present |
| 45Y | Oval and Round | Isolated or grouped in small clusters | Present |

**Table 7: Carbohydrate Fermentation Test of Temperature Tolerant Yeast Strains from Molasses and YPD Broth Medium Based Cultures**

| **Type of Sugar** | Sample | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **20M** | **20Y** | **25M** | **25Y** | **30M** | **30Y** | **35M** | **35Y** | **40M** | **40Y** | **45M** | **45Y** |
| Dextrose | + | + | + | + | + | + | + | + | + | + | + | + |
| Starch | - | - | - | - | - | - | - | - | - | - | - | - |
| Fructose | + | + | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + |
| Galactose | + | + | + | + | + | + | + | + | + | + | + | + |
| Trehalose | + | + | + | + | + | + | + | + | + | + | + | + |
| $\alpha$ Methylglucoside | + | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + |

(+) Positive, (-) Negative

**Fig 1: Molasses Medium kept for aerobic and anaerobic fermentation**

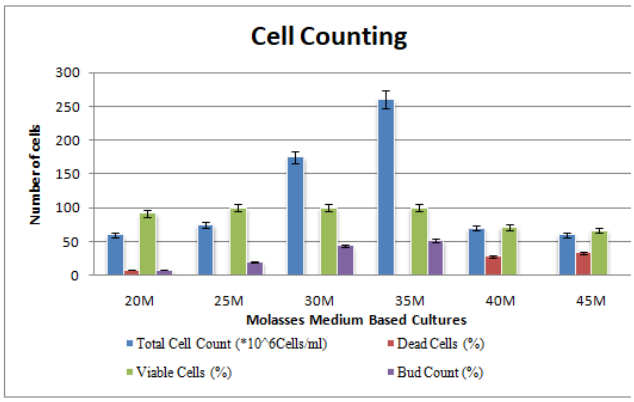



**Fig 2: Cell Counting of Molasses Medium based Cultures grown at different temperatures**

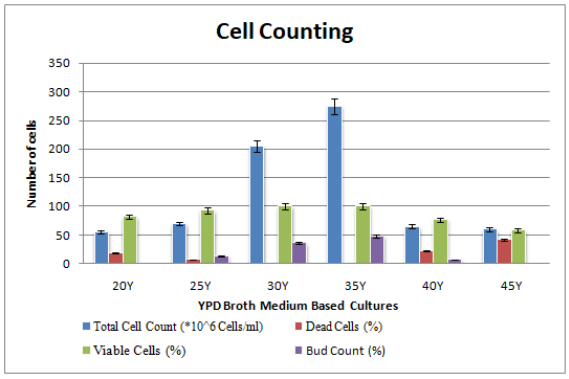



**Fig 3: Cell Counting of YPD Broth Medium based Cultures grown at different temperatures**

**Fig 4: Growth of Yeast Cultures from Molasses Medium based cultures on YPDA Medium**

**Fig 5: Growth of Yeast Cultures from YPD Broth Medium based cultures on YPDA Medium**

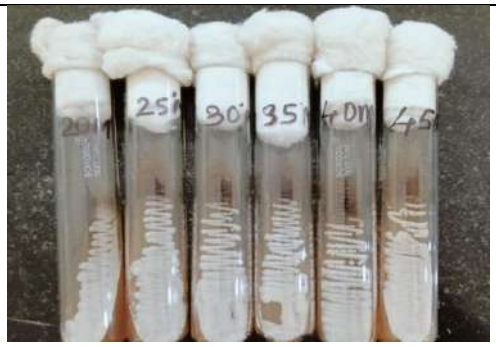


**Fig 6: Pure Culture of Yeast Cultures from Molasses Medium Based Cultures**

**Fig 7: Pure Culture of Yeast Cultures from YPD Broth Medium Based Cultures**

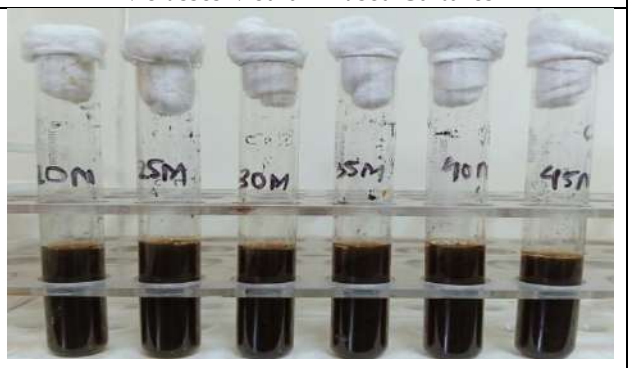
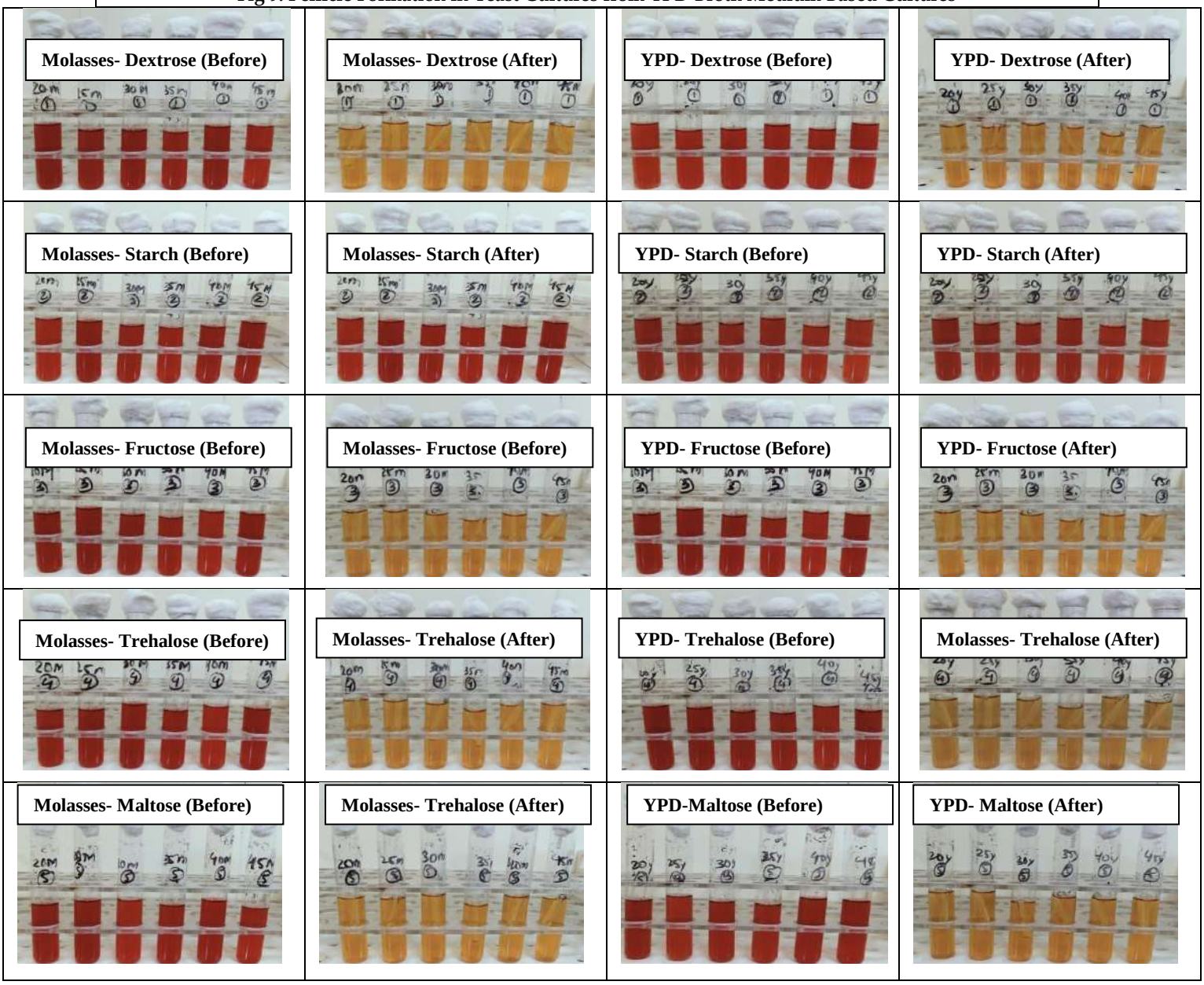



**Fig 8: Pellicle Formation in Yeast Cultures from Molasses Medium Based Cultures**

**Fig 9: Pellicle Formation in Yeast Cultures from YPD Broth Medium Based Cultures**



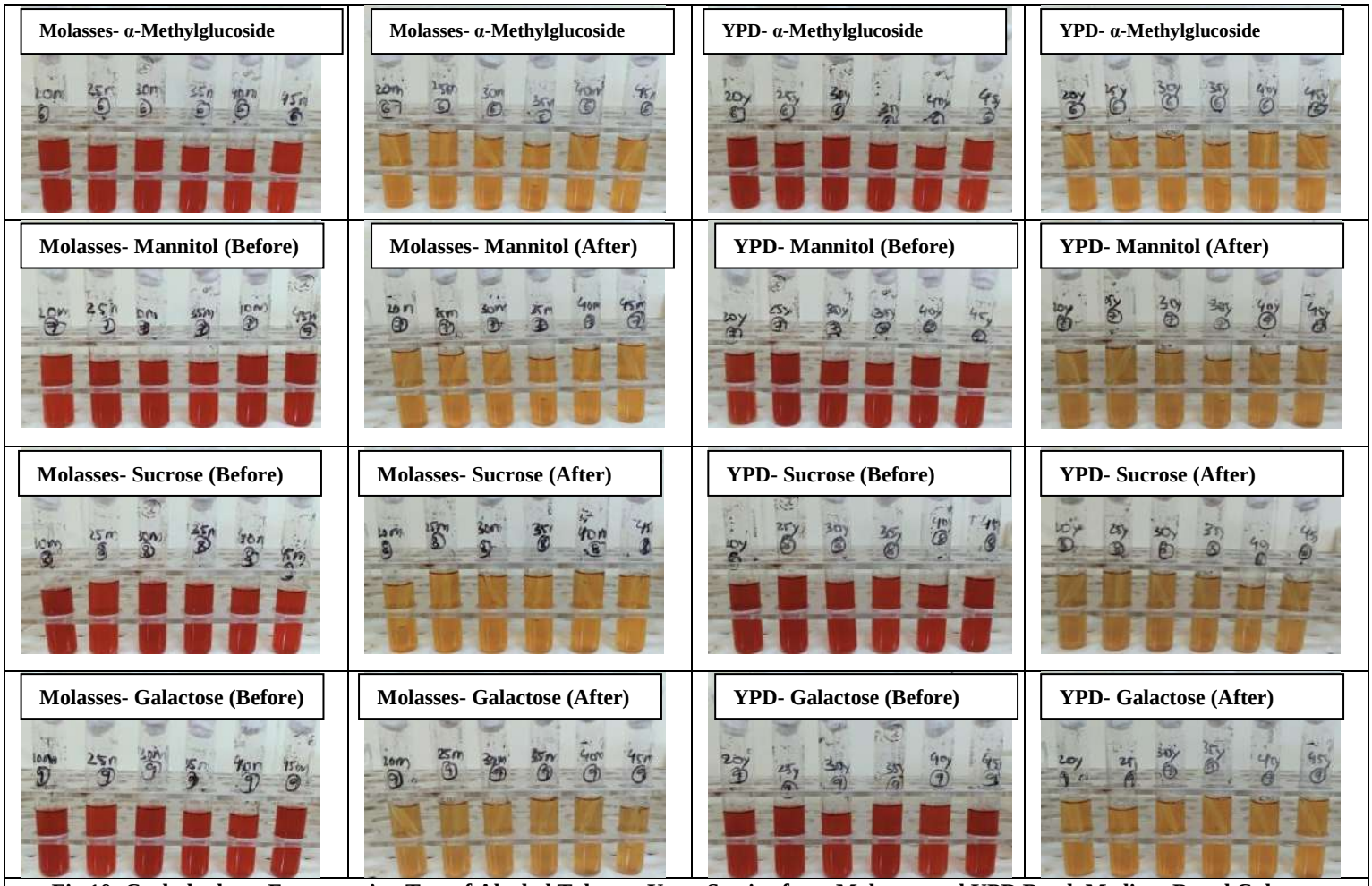



**Fig 10: Carbohydrate Fermentation Test of Alcohol Tolerant Yeast Strains from Molasses and YPD Broth Medium Based Cultures**

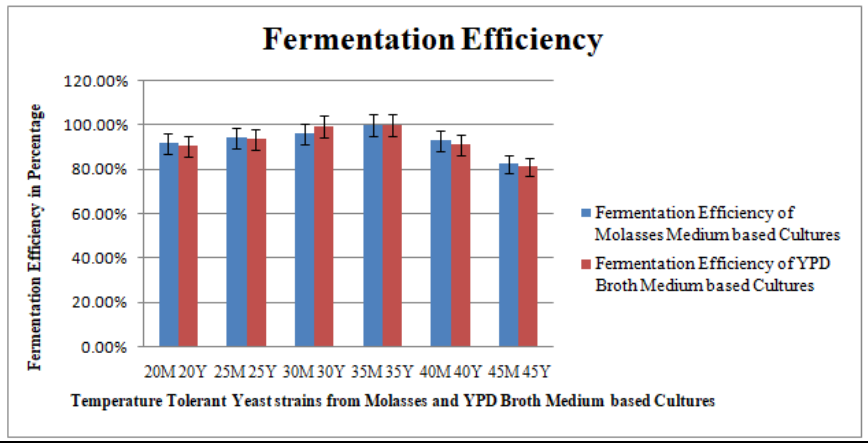



**Fig. 11: Fermentation Efficiency of Yeast strains from Molasses Medium and YPD Broth Medium based Cultures**

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**RESEARCH ARTICLE**

# Generational Differences with Respect to Perceived Social Support, Self-Esteem and Resilience

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**ABSTRACT**

Generational gap and differences have always been a topic of research. These intergenerational differences are prominent and can be observed very distinctly, be it in terms of physical, social, emotional, and/or psychological abilities. This study aimed to investigate a significant difference between Generation X and Generation Z in terms of self-esteem, resilience, and perceived social support. The survey incorporated basic questions about the participants such as their names, ages, and gender. It most importantly consisted of 3 self-report measures namely the Rosenberg Self-Esteem Scale, The Multi Social Scale of Perceived Social Support, and the Connor Davidson Resilience Scale. 172 participants of Indian nationality participated in this study. Out of which 86 belonged to Generation X and 86 to Generation Z. Independent samples t-test was used to analyze the data. The findings demonstrated a significant difference between the two generational cohorts in terms of self-esteem, resilience, and perceived social support.

**Keywords:** Gen X, Gen Z, Self-esteem, Resilience, Perceived Social Support

## INTRODUCTION

The word 'generation' is commonly used when denoting an entire age group of people and locating individuals within a historical time frame. Many social scientists have tried to define a generational cohort in their ways. According to Karl Mannheim(1952), a generation can be described as, "A group that is distinctive in any number of respects by having experienced a specific set of social, economic, technological, and/or political circumstances at a formative period in their lives". The term 'generational cohort' is often used interchangeably with terminologies like 'age cohort' or 'birth cohort' in fields like social sciences, marketing, humanities, demographics, etc. A generational cohort is a group of people who were born within a few years of each other into the same historical and social-cultural context and have developed common attributes caused by shared participation in similar life events (Lyons

&Kuron, 2014; Schermerhorn, 2013; Twenge et al., 2010)(Lyons, S., Urick, M., Kuron, L., & Schweitzer, L., n.d.).This research deals with two generational cohorts: Generation X also called Gen X and Generation Z called Gen Z. It tries to understand variables such as self-esteem, resilience, and perceived social support and how they affect these generational cohorts as well as their comparative analysis.

**Generation X (also referred to as Gen X)**

Though there has been a debate about the birth years of Generation X, this generational cohort is thought to be roughly born between themid-1960s and the early 1980s. Generation X individuals grew up facing economic difficulties and uncertainties as well as rapid social change. Hence, they embody a spirit of independence and self-reliance. They tend to be skeptical of authority and institutions compared to other generational cohorts. They have a strong sense of work-life balance and prioritize work-life along with personal enrichment. Generation X is known for their strong work ethic, adaptability, and self-sufficiency (Tulgan, 1996). They face heightened polarization and anxiety due to the uncertainty of societal positions and ambiguous rules (Rymarz, 2006). However, contrary to some portrayals, they are not universally cynical or indifferent (Maschaykh, 2012). Instead, they display a blend of traditional and innovative mindsets, with various age segments within the cohort gravitating toward distinct mentalities (Pishchik, 2020). Research on Generation X has revealed a variety of viewpoints, encompassing both favorable and unfavorable assessments. Mensik(2007) and Maschaykh (2012) both acknowledge the existence of negative stereotypes, with Mensik advocating against oversimplified and restrictive categorizations. However, Tulgan (1996) counters these stereotypes by portraying Generation X as industrious individuals driven by a strong ambition to excel in their careers.

**Generation Z (also referred to as Gen Z)**

Generation Z, born post-1995, constitutes a distinct demographic marked by innate digital proficiency, distinct attributes, and a need for further scholarly exploration (Jayatissa, 2023). Their formative years have been shaped by pivotal events like the aftermath of 9/11, the Great Recession, and the COVID-19 crisis, rendering them a diverse, globally-connected, and mobile cohort (McKee-Ryan, 2021). Within academia, they exhibit adeptness with technology and anticipate access to online educational resources (Zorn, 2017). In professional settings, they manifest unique work ethics and career inclinations, heavily influenced by the impacts of the COVID-19 pandemic (Mahapatra, 2022). They are recognized as digital natives, adept at utilizing technology and engaging with social media platforms (Sakashita, 2020; Jayatissa, 2023). Notably, this generation is celebrated for its diversity, exhibiting a notable level of racial inclusivity (Seemiller, 2015). They are characterized as entrepreneurial, socially aware, pragmatic, and encompassing diverse perspectives (Jayatissa, 2023). Additionally, they are described as intelligent, driven, innovative, accountable, compassionate, and empathetic (Seemiller, 2015). However, despite these commendable attributes, they confront challenges stemming from growing up in an era marked by economic instability and natural calamities (Sakashita, 2020). They often harbor pessimism influenced by economic and environmental concerns (Sakashita, 2020), and demonstrate lower commitment to organizations and long-term endeavors compared to prior generations (Lev, 2022). This cohort exhibits adeptness with technology, relying heavily on social media and experiencing a fear of missing out (Herawati, 2022). They prioritize flexibility and digital work environments, displaying a penchant for pursuing personal aspirations in the workplace (Ruzsa, 2018). These dynamics can present obstacles in the job market and hinder efforts to maintain harmonious work-life equilibrium.

Generation Z grapples with distinctive psychological hurdles stemming from their digital upbringing and the influence of globalization (Ermolova, 2020; Maioli, 2016). Their engagement with virtual reality further molds their psychological makeup, exhibiting diverse degrees of depth and direction (Puchkova, 2017). These elements may pose challenges in their transition into the workforce, as they harbor dissimilar expectations and requirements in contrast to preceding generations (Ermolova, 2020; Maioli, 2016).

**Self – Esteem**

Self-esteem is defined as, "One's positive or negative attitude toward oneself and one's evaluation of one's thoughts and feelings overall in relation to oneself" (Rosenberg,1965).The American Psychological Association (2023)

definesself-esteem as,"The degree to which the qualities and characteristics contained in one's self-concept are perceived to be positive." As anyone can determine, high self-esteem paves the way for better mental health and helps you handle adversity easily. It helps us deal with the stresses of everyday life effectively whether it be from school, work, social circles, etc.(Nguyen et al.,2019).Highself–esteem also prevents and reduces the risk of mental disorders like depression and anxiety and helps us face challenges in life while maintaining a favorable attitude towards ourselves(Henriksen et al., 2017). Low levels of self-esteem are unfavorable to one's self-concept and overall well-being. Someone with a low level of self-esteem has trouble with prioritizing themselves, has an external locus of control, exhibits negative self-talk, thinks very poorly about themselves, has a poor outlook on the future, etc. As mentioned earlier, having a negative sense of self-esteem can cause one to become prone to mental health disorders like Anxiety, and depression. Not only that it can also lead to Internet Addiction (Naseri et al., 2015), social anxiety disorder (Maldonado et al., 2013), and risky behavior (Gartland et al., 2013). Someone with high self-esteem can shake off negative feedback or constructive criticisms very easily while someone with a low sense of self-esteem takes everything personally. This can make them more susceptible to giving up and not facing challenges (Kalvin et al., 2016). The development of self-esteem is largely governed by experiences in an individual's life (Baumeister, 2003). Parenting styles in childhood have a major impact on the self-esteem of a person(Coopersmith, 1967). Unconditional love from parents in childhoodplays a major role in helping a child develop a sense of being respected and cared for. These feelings are carried out later inlife as the child grows older (Olsen et al.,2008). Self-esteem is likely to increase during adolescence and young adulthood, being its highest in middle adulthood(Orthand Robins, 2014).

Research suggestsa significant difference between Gen X and Gen Z in terms of self-esteem(Lyons,2007).Innumerable factors like the rise of social media,and technological advancement are responsible for this major shift between the cohorts. Generation Z is the generation in which social media has been introduced. It has been observed that although Gen Z is skilled in handling social media, they face challenges when it comes to face-to-face interactions, which could affect theirself-esteem. This issue is compounded by the prevalent fear of being left out on social media platforms, known as FOMO, among Gen Z individuals (Herawati, 2022).Social networking sites can influence the self-esteemof individuals by triggering cognitive mechanisms, by providing a space for social comparison, and by internalizing beauty standards (Vogel et al., 2014). Low usage of social mediareported high levels of self-esteem and life satisfaction (Blachnio et al, 2016). Twenge (2012) further noted that younger generations place more importance on extrinsic values like money, image, and fame and there has been a gradual decline in intrinsic values such as community service, charity, self-acceptance, etc. which could also impact self-esteem. These findings suggest societal and cultural shifts may influence different generations' self-esteem. One factor contributing to the prevalence of birth cohort effects on self-esteem is the differential impact of cultural and historical events on individuals depending on their age at the time. Elder's (1979, 1981a, 1981b) research on the Great Depression provides a classic illustration of this concept. He observed that individuals who experienced depression during childhood exhibited distinct outcomes compared to those who encountered it during adolescence.Lower levels of self-esteemhave been linked with an increase in factors like self-consciousness, vulnerability to criticism, the tendency to present false impressions in front of othersas well and the tendency to fantasize are some of the traits and tendencies present that affect self-esteem (G. Elliot,1982). These traits are present in today's generation which brings out lower levels of self-esteem.

**Resilience**

'Resilience'term was popularized by psychologist Emmy Werner in the 1970s and 1980s, who conducted a 40-year study involvinga cohort of Hawaiian children belonging to low socio-economic backgrounds. Her research consisted of risk and resilience among the children. Resilience can be defined as the qualities in a person that help him/her to thrive in adverse situations and can also be considered a measure of stress-coping abilities (Connor-Davidson,2003). The American Psychological Association (APA) defines Resilience as the process and outcome of successfully adapting to difficult or challenging life experiences, especially through mental, emotional, and behavioural flexibility and adjustment to external and internal demands. Both internal and external factors affect a person's level of resilience internal factors like self-assessment, self-regulation, and a positive outlook on life, and external factors like relationships with family, friends' community, and social support systems are important regulators of resilience. (Southwick et al., 2014)

Resilience plays a key role in promoting well-being and mental health as well as physical health. Psychological resilience plays a very important role in promoting mental health well-being as well as physical well-being. Resilient people are more capable of navigating life's challenges, maintaining positive emotions, and recovering from setbacks. They endure life's difficult challenges, have good problem-solving skills, and show self-efficacy (Bonanno, et al., 2004). Resilience can also beassociated with bouncing back after a stressful situation occurs in one's life. When a person encounters a lot of stress it disturbs the external and internal sense of balance. Studies have shown that it is not just stress that affects a person's balance but how the person perceives stressful situations is also an indicator of how resilient a person is (McGonigal, 2016).

Generation Z is also popularly known as the 'Digital natives 'and 'Net generation'. As the name suggests, this generation is and has been going through a lot of Technological advancements and changes caused due to it. Being highly vulnerable to social and economic risks this cohort is considered asprey to the digital predators. Researchers suggest that this affects the resilience of Gen Z negatively (Novkoska, 2018). It is believed that exposure to adverse conditions develops resilience in an individual (Luecken and Gress, 2009).Individuals belonging to Generation Z are perceived to be 'bubble wrapped' due to helicopter parenting (Talmon, 2019), hence they don't face adverse conditions as such and don't build up higher levels of resilience. (Ang et al., 2021).Psychological resilience is determined by an intricate nexus of social, genetic as well as environmental factors. Socio-contextual factors like gender, race, ethnicity, age, exposure to trauma, chronic diseases, stress factors, changes in income, education, and life stresses can impact resilience (Bonanno, 2007). The transmission of psychological resilience between generations is seen to be dependent upon family dynamics as well (Regin et al., 2016). Generation X seemed to have stronger family dynamics and bonds whereas Generation Z lacked in these terms.Diverse challenges like transition to adulthood and economic hardship (Conger, 2002) can affect the levels of resilience between the two generations.Demographic features like education level, sex, and income level lead up to 11% of variation in resilience. The individuals who have suffered childhood maltreatment contributed to the anticipation of resilience by 2%.Individuals who have encountered childhood mal-treatment,and individuals with lower levels of income and education demonstrated lower levels of resilience (Campbell, Forde and Stein, 2009).

**Perceived Social Support**

Perceived Social support refers to one's understanding and perception of the emotional, tangible, and informational support they receive from their family members, friends, and others. (Zimet et al, 1988). Social support is a multidimensional concept, referring to the social and psychological support an individual receives or perceives as available to them from family, friends, and their community (Awang et al., 2014; Zimet et al., 1988).

Perceived social support refers to the perception that support would be available if needed (Day & Livingstone, 2003) and comprises emotional and instrumental support (Trepte&Scharkow, 2016).Ample evidence suggests thatsocial support plays an important role inpromoting psychological health and mental well-being (Cohen, 2004)and that social support gives a sense of protection in psychological terms.(Haddadi&Besharat, 2010). Higher levels of social support are associated with an enhanced sense of psychological well-being (Glozah, 2013; Poudel et al., 2020). Social support has consistently been shown to affect mental health disorders like depression, and anxiety and reduce their effects significantly (Ibrahim et al., 2013). Life Satisfaction was highly correlated with social support from family and friends (Harikandei, 2017) and lower loneliness (Lee & Goldstein, 2016). According to Day and Livingstone (2003), perception of one's social support network and resources has majorly greater coping effects than if theyreceive the support and exerts a stronger effect on mental health than actual social support received (Hefner & Eisenberg, 2009). The belief that one has access to social support can act as reassurance in times of need acts as a protective barrierin times of stress,and can make a person happy and makes them have a positive outlook toward life (Winemiller et al., 1993).Individuals' perception of support is determined strongly by actual interpersonal communications as reported by significant others, moderately by the recipients' negative outlook bias, and weakly by their anxiety and depression as indicators of their poor mental health (Vinkour, Schul, and Caplan, 1987). Social support was significantly related to positive dimensions of subjective mental well-being such as gratification, self-esteem, and happiness but not to its negative dimensions such as uncertainty, strain, and vulnerability, and was also significantly related to individuals'

perceived ability to indulge inpositive life events, but not to their perceived ability to avoid or cope with negative events (Meehan, Durlak and Bryant, 1993).

Research conducted on the perception of support in Generation X and Generation Z has revealed significant insights. Reinikainen (2020) discovered a positive correlation between organizational attentiveness on social platforms and increased trust and perceived advantages in the content disseminated by corporations and entities. Goodwin-Smith (2019) emphasized the significance of constructive interpersonal bonds in optimizing resilience and well-being among marginalized teenagers and young adults. Nautiyal (2017) conducted a comparative analysis of the perceived support among adolescents dwelling in urban and rural environments, underscoring its pivotal role in fostering a sense of certainty amidst varied circumstances. Lastly, Feather (2012) uncovered a favorable relationship between values associated with transcending oneself and backing for communal initiatives, implying their potential influence on the readiness to rectify prior injustices. Research into the perceptions and values of Generation Z suggests the possibility of lower levels of perceived social support. Leslie (2021) distinguished three unique subsets within Generation Z, each with different priorities regarding the workplace, implying potential variations in their requirements for social support. Rola (2018) revealed Generation Z's relatively diminished empathetic values, potentially affecting their capacity to both offer and receive social support. McIver (2001) observed a growing sense of reduced backing for public services among young individuals, which might extend to social support systems. Hathaway (2022) underscored the potential influence of digital media on Generation Z's social interactions, which could further shape their perceptions of social support.

The present study deals with Generation X and Generation Z in terms of self-esteem, perceived social support, and resilience. It also sheds light on factors that might affect both the generational cohort's variables mentioned. We often experience differences in generational cohorts. We are all the same but we have experienced our lives differently. For example,Generation Z's parents find it difficult to handle technology whereas Generation Z has found out their way of living through technology with ease. The previous generations were physically active and demonstrated betterlevels of fitness than the later generational cohorts.Transformations like these are thought to be the foundationof why so many changes, physical and psychological, arose. This study analyses whether Generation X and Generation Z have or do not have a significant difference in self-esteem, resilience, and perceived social support.So many attributes like technological advancements, social media, parenting styles, the COVID-19 pandemic, family dynamics, etc. are the root causes these variables such as self-esteem, resilience, and perceived social support are affected inter-generationally and are chosen to be studied.

**Objectives of the study:**

- To assess levels of self-esteem between Generation X and Generation Z.
- To assess levels of resilience between Generation X and Generation Z.
- To assess levels of perceived social support between Generation X and Generation Z.

## REVIEWS OF LITERATURE

Kvintova and Cakirpaloglu (2019) published a paper on "Self Esteem, Social Network Use and Life Satisfaction among College Students of Generation X Y, and Z." This study aimed to correlate the level of self-esteem and life satisfaction to social network use inter-generationally. There was no correlation found between theself-esteem and amount of time spent on social media. It was found that Generation Z was inclined to be more satisfied in two areas: leisure time and sexuality. They use social media three times compared to Gen X and Y. Also, they had more number of friends on social media. Soest (2016), in his study, "The Development of Global and Domain-specific Self-esteem from Age 13 to 31", examined the development of global self-esteem and self-esteem in 6 domains across adolescence and young adulthood. It demonstrated that later generations exhibit high self-esteem when it comes to social self-esteem and appearance.

Twenge and Campbell (2001) have explored how self-esteem has changed over different age groups and birth cohorts over time and found that younger generations born in the 1980s and 1990s had higher levels of self-esteem than the previous generations such as those born in the 1960s and 1970s. R Van Woensel (2018) researched, "Generation Z: A Generation of lower self-esteem". The findings suggested that Generation Z showcased lower levels of self-esteemthan the previous generationsdue to various factors like social media influences, academic and career pressures, and the ongoing culture of comparison among other factors. Sethi (2023) conducted a study on, "The role of Perceived Social Support in self-esteem and resilience among young adults."The results showed that perceived social support correlates positively with resilience and self-esteem among young adults. No significant gender differences were found. Angeles and Perkins (2023)explored howintergenerational differences affect self-efficacy coping and resilience of members of a family during a crisis (covid pandemic).The results portrayed that resilience and a sense of coherence are interrelated but self-efficacy coping shows no disparities between the generations. Frankczok Kuczmowska and Barbara Superson (2023), in their research, "Resilience in the Context of the Socio-Political Situation of young adults in Europe", displayed contrasting results to the current study. This research focused on how amidst the social and political situation of Europe young adults are showing the highest levels of resilience than any other age group.

**Hypotheses:**

- There will be a significant difference between Generation X and Generation Z in terms of self-esteem.
- There will be a significant difference between Generation X and Generation Z in terms of resilience.
- There will be a significant difference between Generation X and Generation Z in terms of perceived social support.

## METHODOLOGY

**Participants:**

The population consisted of Indian citizens. Individuals belonging to Generation X and Generation Z were used as samples in this study. Stratified random sampling was used as the sampling technique. Separate age groups of 18 to 28 years for Generation Z and 43 to 58 years for Generation X were selected for this research. The following generational cohorts were excluded from this study:

a) Baby Boomers (1946-1964): 59-77 years old.

b) Millennialsor Generation Y (1981-1996): 29-42 years old.

c) Generation Alpha (early 2010s- 2025): 0-10 years old.

A total of 172 responses were collected for the research. 86 responses were collected for Generation X and 86 were collected for Generation Z. Demographic features like name, age, and gender were collected as well. 61. 5% of the individuals who participated in the study were females whereas 38. 5% were males.

**Tools**

a) The Rosenberg Self-EsteemScale: Morris Rosenberg developed the scale to measure one's self-esteem. It contains 10 items that measure both positive and negative feelings about the self. A 4-point Likert scale is used to answer the items with options being Strongly agree, Agree, Disagreeand Strongly Disagree. Strongly agree carries the value of 4 whereas strongly disagree carries the value of 1. Higher scores indicate high self-esteem and low scores indicating low self-esteem. As this scale uses Guttman'sscalogram, it includes reverse scoring as well. The scale has a high reliability as Cronbach's Alpha for the scale was reported at a high Alpha equal to 0. 894. It demonstrates concurrent, predictive, and construct validity.

b) Connor-Davidson Resilience Questionnaire: The Connor Davidson Resilience Scale was constructed by Katheryn M. Conner and Jonathan R. T. Davidson. The scale aims to measure resilience or the ability to bounce back after a traumatic event or stress-inducing situation. The scale has 2, 10, and 25-item versions. In this research 25 item version has been used. It is a 5-point Likert scale ranging from 0 to 4. 0 being not true at all and 4 being true nearly all of the time. The scores range from 0 to 100 with higher scores indicating high levels of resilience and

lower scores indicating lowlevels of resilience. The CD-RISC demonstrates good reliability with Cronbach alpha being 0. 88. Construct, convergent, and predictive validities have been established.

c) The Multi-dimensional Scale of Perceived Social Support: Perceived social support refers to one's understanding and perception of the emotional, tangible, and information support they receive from their family members, friends, and others (Zimet et al.,1988). The multi-dimensional scale of perceived social support (MSPSS) was created byZimet, G.D., Dahlem,N.W., Zimet,S.G., and Farley, G.K.(1988). It was developed to measure perceived social support, particularly from 3 sources that are friends, family, and significant other. It includes 12 items and is a 7-point Likert scale ranging from 1 to 7. Very strongly disagree is 1 and very strongly agree is 7. The mean scores range from 1 to 7 with low scores indicating low levels of perceived social support whereas high scores indicating high levels of perceived social support. The scale shows a high reliability with Cronbach's Alpha being equal to 0. 85. A good Construct validity has been established.

**Procedure**

Assessment was done by circulating Google form. The link was sent to family members, friends, relatives as well as acquaintances. The form consisted of five parts, the first part included the information about the research, the second part included the demographic details, and the third one consisted of the Rosenberg self-esteem scale (RSE). The fourth part included the Multidimensional scale of perceived social support (MSPSS) and the fifth one consisted of the Connor Davidson resilience scale (CD-RISC). Instructions were given before each section on how to solve the questions. All the observations received in the form of responses were properly collected and arranged in a tabular format for statistical analysis. It was ensured that confidentiality would be maintained and the data collected from them would be used for purposes concerning research only.

**Statistical Analysis**

After the data collection, scoring was done. The data was analyzed by using SPSS software. Independent sample t-test is for the analysis.

## RESULT AND INTERPRETATION

All the collected data was subjected to SPSS software in order to obtain the results.An Independent sample t-test was used to analyze the difference between resilience, self-esteem, and perceived social support between Generation Z and Generation X.

**Self-esteem and generational cohort:**

Results indicate a significant difference between Generation X and Generation Z regarding self-esteem. Generation Z (*M[SD]*= 28.74[3.717]) reported significantly (*t*=4.325) lower levels of self-esteem than Generation X (*M[SD]*= 31.36[4.360]).

**Perceived Social Support and generational cohort**

Results indicate a significant difference between Generation X and Generation Z regarding perceived social support. Generation Z (*M[SD]* =5.13[1.335]) reported significantly (*t*=3.326) lower levels of perceived social support than Generation X (*M[SD]*= 5.71[0.919]).

**Resilience and generational cohort**

Results indicate a significant difference between Generation X and Generation Z regarding resilience. Generation Z (*M[SD]* =69.74[12.217]) reported significantly (*t*=3.176) lower levels of perceived social support than Generation X (*M[SD]*= 75.74[11.394]).

## DISCUSSIONS

It is known fact that changes happen from generation to generation. This investigation aimed to find these differences with respect to self-esteem, perceived social support and resilience. Results revealed significant differences between Generation X and Generation Z in all mentioned variables. Generation X is showing high level of self-esteem, resilience and perceived social support as compared to Generation Z.

In self-esteem, various researches shed light upon how Generation Z has lower levels of self-esteem than Generation Y. Various factors such as helicopter parenting, social media, economic uncertainty, etc. are responsible for a lower self-esteem. One possible explanation for Generation Z's low self-esteem might be the fact that Generation Z has grown up in an Era dominated by social media which propagates perfectly showing our lives even when they are not. Djedovic` et al. (2021) in their study, "Post Millenials: Meet Generation Z", highlighted that Generation Z is the first generation to be born after the surge of the internet. As a result of being born in a world in control of social media, caring about the number of likes they will get and the amount of followers they have. Their happiness will depend upon the instant gratification they will get as soon as they see that someone has liked their story or post. Since their life is based upon being in the public's eye through social media, their appearance matters a lot to them. This makes them an egocentric generation with no empathy. Being so conscious about looks and appearance in social media and real life makes this generation insecure and have a low sense of self-esteem. The rise of social media has also paved the way for building unrealistic expectations, the inability to have face-to-face conversations and over-dependence on social media. Parenting styles as mentioned earlier also add as a contributing factor to low levels in self-esteem. Bee (2017) researched parenting styles and successive generations which aimed to investigate the relationship between self-esteem and parenting styles across three generational cohorts consisting of baby boomers, Generation X and Generation Y. The hypothesis was stated that the permissive parenting style would increase with successive generations and that would lead to a decline in self-esteem. One plausible explanation was given that whenever the child is subjected to a permissive parenting style and given unconditional positive regard completely, they search for validation externally as they have a particular self-image formed but later, he/she realizes that the external world does not treat everyone equally. Always getting unconditional positive regard from parents as a child and getting positive comments and appreciation even after doing average/mediocre tasks can lead to an initial boost in self-esteem as a child but can lead to false and high expectations from the outside world as well. This generation also faces a lot of economic uncertainty which leads to minimal job opportunities and a low sense of financial security for the individuals belonging to this generational cohort. The proposed hypothesis that there will be a significant difference between Generation X and Generation Z in terms of self-esteem is supported by results.

In case of perceived social support, Generation Z indicated lower levels of perceived social support than Generation X. A lot of research has been done that supports the hypothesis. A plethora of factors have contributed to lower perceived social support levels. Factors like age, gender, educational qualifications, lower usage of social media, etc. have contributed to these findings. A study by Chen et al. (2014) called, "The perception of social support among U.S. Chinese older adults: findings from PINE Study" aimed to examine the correlates and perceptions of negative and positive social support among U.S. Chinese older adults. The findings demonstrated that being of a younger age and being male correlated positively with negative social support or lower levels of perceived social support. Older adults portrayed high levels of perceived social support and it is dependent on factors like higher health status, better quality of life, higher education levels, gender (Female), and improved health over the past years were associated with higher levels of perceived social support. Another research by Coventry et al. (2004) focused on how perceived social support differs across age and sex. The sample consisted of participants aged between 18-95 years. It was found that perceived social support varied across different age groups. Older adults belonging to the previous generational cohorts have higher levels of perceived social support than the younger adults of the later generations. One of the reasons why Generation Z has lower levels of perceived social support might be because of the poor quality and a lack of close-knit relationships, be it, with friends, partners, parents, etc. They engage in superficial relationships that do not have any kind of emotional depth or emotional intimacy. Reasons for this can include the

emergence of social media which results in a lack of face-to-face communication, loneliness, and genuine social communication. Luong, Charles and Fingerman (2011) in their study, "Better with age: Social relationships across adulthood", emphasize why older adults have significantly reported greater satisfaction with their social connections compared to younger individuals. Older adults who belong to the previous generations have better bonding with their children, good and closer friendships, and long-lasting marriages which overall leads to a higher sense of perceived social support amongst the older adults. It was also observed that interpersonal clashes often decline with age. Relationships that are less problematic and ambivalent were seen than those of the younger adults. Interpersonal stressors were also reduced, such as agreements and disagreements, than younger adults. An interesting finding demonstrated that the ability to navigate through difficulties in a relationship increases with age. The proposed hypothesis that there will be a significant difference between Generation X and Generation Z in terms of perceived social support is accepted and supported by results.

In case of resilience, Generation Z has shown lower levels of resilience than Generation X. Resilience, which is defined as the successful ability to adapt to adversity and stress, is affected by personal competence, self-acceptance, and the capacity for meaning and improvisation. The global pandemic like COVID-19 has affected resilience of individuals across the globe. Smith (2023) research explored the undergraduate students' resilience during the Covid 19 pandemic on Generation Z. 27 students participated in this study to share their experiences in focus groups. The findings suggested that the pandemic harmed the mental health and resilience of Generation Z students. It was implied in this study that Generation Z has lower levels of resilience than any other generation. COVID-19 compromised a lot of factors like self-esteem, self-efficacy, and bonding, a sense of belongingness, emotional health, and subjective well-being which promote to one's overall resilience. Hence, the levels of resilience had been reduced. Social isolation due to COVID-19 has also paved a way for feeling lonely which in turn has affected resilience as well. Sethi (2023) conducted a study on young adults and the point of the study was to know the role of perceived social support in promoting resilience and self-esteem among young adults. From the result of the study, the present hypothesis, that Perceived Social support portrays a positive relationship between self-esteem and resilience, is accepted. It means that the self-esteem of a person is directly proportional to the perception of social support; they can quickly handle things and cope with the problem at hand, more than those who perceive a low amount of social support and differences according to their gender. There exists a perfect correlation between the three variables. In the case of Gen Z, these factors are likely to be lacking due to the negative impact on their formation, for instance, economic instability and the adaptation to adult life. This implies that personal and environmental factors might play equal roles in the perception of Gen Z's low resilience. As mentioned earlier, low levels of perceived social support are seen in Generation Z individuals. Low levels of perceived social support are directly proportional to loneliness and the feeling that no one will be there in times of need. Hence, it decreases the capacity to withstand tough conditions and arise from them. Parenting is also one of the important factors. Helicopter and permissive parenting are the current trends in parenting and Generation Z kids are subjected to such parenting styles. As mentioned earlier these are a cause for low self-esteem. It can also be a source for lower resilience levels. Overly protective parenting can cause children to never face any kind of adversities in their lives and can explain why Generation Z is lacking in resilience. COVID-19 has also contributed to a major decrease in the resilience levels of individuals all over the world. Generation Z which already has lower levels of resilience has faced prominent repercussions for struggling with social isolation and dealing with loneliness as there was no social contact. The proposed hypothesis that there will be a significant difference between Generation X and Generation Z in terms of resilience is supported by results.

## CONCLUSION

This investigation concluded with the findings that all proposed hypotheses are accepted and there are significant differences between two Generations (X and Z). This study opens a way for future scope regarding aspects like increasing resilience, self-esteem, and perceived social support in various settings such as organizational, educational

and therapeutic. The research paves the way for getting to know the generational trends present in both generations and enhances our way of getting to know them.

**Limitations**

- The technical challenges of data collection would have been eliminated if the data was collected in person and face to face and probably a larger sample would have been collected.
- Qualitative responses about every individual from each generational cohort should have been collected to know more about the reasoning behind the answers they chose and it would have given an insight into the things they face because of belonging to that specific cohort.
- More demographic details of the participants should have been collected to carry out further statistical analyses and to get to know more about the characteristics of the population.

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**Table 1. Showing Mean, SD and t-value**

| Variable | Generational Cohort | N | Mean | Std. Deviation | t-value |
|---|---|---|---|---|---|
| Self-esteem | Gen Z | 86 | 28.74 | 3.717 | 4.325* |
| | Gen X | 86 | 31.36 | 4.360 | |

*p<0.05

**Table 2.: Showing Mean, SD and t-value**

| Variable | Generational Cohort | N | Mean | Std. Deviation | t-value |
|---|---|---|---|---|---|
| Perceived Social Support | Gen Z | 86 | 5.13 | 1.335 | 3.326* |
| | Gen X | 86 | 5.71 | 0.919 | |

*p<0.05

**Table 3.: Showing Mean, SD and t-value**

| Variable | Generational Cohort | N | Mean | Std. Deviation | t-value |
|---|---|---|---|---|---|
| Resilience | Gen Z | 86 | 69.74 | 12.217 | 3.176* |
| | Gen X | 86 | 75.74 | 11.394 | |

*p<0.05

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**RESEARCH ARTICLE**

# Impact Assessment of Static Waterbodies in Peri-Urban Areas: The Case of Kakori Town, Lucknow

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**ABSTRACT**

Peri-urban ecosystems and societies depend on static waterbodies. Static water bodies in Lucknow's peri-urban area, particularly Kakori Town, are examined in this study. The study uses field surveys, remote sensing, and socio-economic studies to investigate the biological and hydrological dynamics of Kakori Town's static waterbodies. This studyanalyses the lake based on the identified parameters (Landuse and Population density, Encroachment, community use, pollution, community participation, excavation activity, recreational or tourism activities and institutional role). It also examines natural and anthropogenic influences on static waterbodies. The research identifies stressors to inform sustainable management techniques and policy measures to mitigate their negative effects and strengthen these vital ecosystems. Policymakers, urban planners, and local communities may use these insights to safeguard these crucial natural resources and promote sustainable development in peri-urban areas like Kakori Town.

**Keywords:** Impact assessment, Static water bodies, Lucknow, Peri-urban areas, Kakori town

## INTRODUCTION

Urbanization has been accelerating globally, particularly in developing nations, and this trend is expected to continue. An estimated 600 million people have joined the global urban population in the past decade, accounting for about two-thirds of the total population growth (Ritchie et al., 2024). Currently, there are twenty-one megacities worldwide, each with populations exceeding ten million, with seventeen located in developing nations (UN-

HABITAT, 2020). In India, urbanization accelerated post-independence due to the shift to a mixed economy, fostering private sector growth. According to the World Bank, the urban population in India increased from 11.4% in the 1901 census to 28.53% in the 2001 census and reached 34% by 2017. A United Nations survey projects that by 2030, 40.76% of India's population will be urban (Bhagat, 2018). By 2050, India, along with China, Indonesia, Nigeria, and the United States, will lead the global urban population growth (World Bank, 2011; UN, n.d.).

Rapid urban expansion in India has led to the depletion of static water bodies due to pollution, waste disposal, and urban encroachment. Consequently, urban areas face groundwater depletion, flooding, and water scarcity, adversely affecting quality of life and access to essential services (Beura, 2017). To address these issues, water security must be a national priority, requiring efficient management and stringent regulations, along with public participation in water conservation (Mundoli et al., 2015). In Lucknow, the number of static water bodies has decreased from 964 in 1952 to 494 in 2006, due to urbanization. The water quality upstream has deteriorated under the pressures of rapid urbanization, population growth, and urban sprawl (Prabha Baiswar et al., 2022). The Gomti River, fed by groundwater and its tributaries, has seen a 35-40% reduction in discharge, becoming one of the most polluted rivers in the country. Factors such as biotic pressure, reduced ecological flow, siltation, encroachment, and heavy metal contamination have contributed to this degradation, threatening the sustainability of Lucknow's water resources (Rai & Singh, n.d.). The peri-urban area of Lucknow, characterized by a mix of rural and urban features, faces challenges related to land use and infrastructure development due to economic growth and demographic pressure (Rawat et al., 2022; Mishra et al., 2024).

## MATERIALS AND METHODS

**Research Method**

The objective of this research article is to examine the effects of urbanisation on static water bodies in peri-urban regions of Lucknow, specifically those urban areas situated between the 2021 and 2031 planning boundaries as delineated in the Lucknow Master Plan 2031. The criteria for selecting static water bodies for analysis purposes are as follows: they must have a minimum surface area of 1 acre and be located within 10 metres of the urban settlement. Static bodies of water within or in close proximity to urban settlements at an altitude of 10 metres have been chosen for the purpose of analysis so that a reconnaissance survey and satellite image can provide a more comprehensive understanding of the water body's local context. The static body of water was additionally required to have a minimum surface area of 1 acre as a secondary criterion. Hence, within the vicinity bounded by the planning boundaries of 2021 and 2031, Kakoritown of Lucknow district stands as the sole remaining settlement worthy of examination, comprising a collection of five static water bodies chosen for analysis. The research utilised cartography based on Geographic Information Systems (GIS) to identify the factors that contribute to the depletion of static water bodies situated within or in close proximity to the boundaries of the KakoriTown. The lake boundaries were established through the utilisation of Google Imagery data pertaining to the years 2011 and 2023, correspondingly. Subsequently, data has been acquired predominantly from a reconnaissance survey concerning the land utilisation in close proximity to the subject site, with a proximity of 100 metres. Following this, the KakoriTownLanduse map was created. Also, the areas of traced static water bodies for the years 2011 and 2023 were computed using ArcGIS. The application of this technique enabled a direct comparison and computation of the depletion state of the static water bodies. Additionally, the aetiology of these depletions has garnered considerable attention.

Then, a comprehensive assessment has been conducted on a lake-by-lake basis on the identified parameters (Mishra et. al, 2024). Those identified parameters were Land Use and Population Density, Encroachment, Community Use, Pollution, Community Participation,Recreational or Tourism Activities, and Institutional Role respectively. User perception analysis has been done for all the individual 5 lakes and the scoring were done by the locals around the lake, from 1 to 5 ( 1 is the least concern 5 is the major concern ). The sample size of the user perception is 30. Then the identified parameters were assigned weightages by using AHP , there after normalization was done for the accuracy,

and hence rankingis devoted to each of the parameters for individual lakes. ( 1 is the major concern 7 is the least concern ).

**Site Selection**

As the most populous state in India and the capital of Uttar Pradesh, Lucknow is representative of the majority of Indian metropolises. Lucknow's growth trajectory remained predominantly organic from 1981 to 1991. However, beginning in 1991, the pattern abruptly shifted towards ribbon development, which benefited from accessibility and transit-oriented development.Theperi-urban and rural regions encircle the city as a result of the city's burgeoning urban population and high migration rate. The urbanised region is presently en-route to Amausi Airport in the southeastern direction, via the Lucknow-Kanpur Highway, Lucknow Raebareli Road, and Lucknow Hardoi Road(Kumari, 2015). The temporal and spatial dimensions of the land use havechanged significantly.Population growth has been consistent in the city due to natural expansion, large-scale migration, and the incorporation of peri-urban regions(TCPO, 2020).

From 1901 to 2011, the population of Lucknow Urban Agglomeration (UA) increased from 2.5 lakh to 28.17 lakh. According to the predictions outlined in the Lucknow Master Plan 2031, the peri-urban regions of Lucknow are anticipated to experience a decadal growth rate of 117.55 percent in 2031, up from 24.35% in 2011 and 27.71 percent in 2021(DoCO UP, 2011). Within the 2031 planning boundary of Lucknow, the number of lakes and ponds decreased from 86 in 2011 to 78 in 2023. Table 2 indicates that urbanization has had a more significant impact on peri-urban areas, where the number of water bodies declined from 66 to 59 between 2011 and 2023. In comparison, the Lucknow Municipal Corporation (LMC) areas experienced a decrease from 20 to 19 water bodies. Furthermore, the total area of water bodies in peri-urban areas decreased by approximately 11.12%, whereas in the LMC areas, it decreased by 7.15%. This suggests that water bodies in peri-urban areas are more vulnerable to the effects of urbanization compared to those within the city areas, which receive more focused preservation efforts.

**Impact of Urbanisation on Static Water Bodies in peri-urban areas of Lucknow**

Many of the lakes that comprised the capital of Awadh in 1900 have been converted into human settlements over the course of the last century. Based on a survey carried out by the Lucknow Municipal Corporation, the quantity of reservoirs in the city experienced a decline from 964 in 1952 to 494 in 2006. The quantity experienced a decline to 494 in 2006, with reclamation rendering the majority of them unidentifiable(IndiaTV, 2019; Siddiqui, 2018; Verma, 2016). Additionally, it has been noted that the majority of bodies of water are clogged with solid refuse, debris, and sewage, among other substances, which diminishes their carrying capacity. Between 2011 and 2023, the number of lakes in the peri-urban regions of Lucknow decreased from 66 to 59, representing a significant reduction of 11.12% in area(Goel et al., 2018).

**Assessment of the Static Water Bodies in the Kakori Town**

The purpose of this research article is to investigate the effects of urbanization on static water bodies in the peri-urban regions of Lucknow, specifically those urban areas situated between the 2021 and 2031 planning boundaries as delineated in the Lucknow Master Plan 2031. The criteria for selecting static water bodies for analysis are as follows: they must have a minimum surface area of 1 acre and be located within 10 meters of urban settlements and Kakori town fulfils all the requirements for selection. Kakori town is located in the Lucknow district of Uttar Pradesh in India. It is a peri-urban area, meaning it is on the outskirts of the city and has a mix of urban and rural characteristics. The town is known for its historical significance and is home to various industries and agricultural activities.Kakori was declared as town in 2001(Team Digital, 2014).The total area of Kakori town is 110.4 hectare.The Kakori Nagar Panchayat is a local governing body in the Kakori town in Lucknow, Uttar Pradesh(*Maps, Weather, and Airports for Kakori, India*, n.d.; TOI, 2011).It is tasked with the provision of fundamental infrastructure and services to the town's inhabitants. Given that the population density of Kakori town is considerably greater than that of Lucknow Municipal Corporation, evaluating the state of static water bodies in Kakori town becomes more justifiable(DoCO UP, 2001).

## RESULTS

As stated previously, five selected static water bodies of Kakori town have been analysed based on those seven parameters mentioned earlier. They are listed in Table 4. Lake wise depleted area value and their major causes for their depletion are being analysed as follows:

**Salariya Lake**

The lake is in ward no. 8 of KakoriTownand is surrounded by two wards i.e. ward no. 11 and ward no. 2. The entire lake is entitled to lease from last 25-30 years. The total area of this lake is 2.1 acre.

**Land Use and Population Density**

The Lake is surrounded with residential areas, vacant land, public/semi-public area (primary health centre) and agricultural land. Ward no. 8 is 1.5 times(127PPH), Ward No. 2 is almost 4 times(326PPH), whereas ward no. 11 is 2 times denser(168PPH) then the Lucknow Municipal Corporation (LMC) population density (85PPH) in 2011.

**Encroachment**

The lake underwent a reduction in area over a ten-year period, from 2.57 acres in 2011 to 2.1 acres in 2023, an equivalent decrease of 0.47 acres. The constructed area surrounding the water body, with a 10-meter buffer zone, is 0.53 acres. Open space is 1.28 acres on the periphery (10-meter buffer zone around the body of water). In contrast, the ratio of built-up to open space in the vicinity of an aquatic body (10-meter buffer zone) is 1:2.41. At a distance of 100 metres from the lake's shoreline, the developed region encompasses 6.52 acres. The extent of open space within the lake's influence area, which is 100 metres from the lake's shoreline, is 12.36 acres. The open space ratio in the lake's influence area, which is 100 metres from the lake's shore, is 1:1.89.

**Community Use**

The poor water quality caused by the excessive sewage discharge into the lake discourages any community use of the lake during the summer, with the exception of commercial fishing and baking earthen wares. Seasonal commercial fishing is conducted for five to six months annually. The remaining socio-economic activities and community uses, including personal hygiene, clothing cleansing, idol immersion, angling, irrigation, animal bathing, cremation, and the Mundan ceremony, are not being observed.

**Pollution**

The pollution in the vicinity of the lake has a detrimental effect on the health of the lake's inhabitants and has also degraded the water quality. The sources of pollution in the vicinity of the lake include untreated domestic sewage discharge, scat, and the dumping of municipal solid refuse along the lake's shoreline. Aside from these, certain types of waste are not being discovered along the lake's shoreline, including agricultural residue, immersion of religious or cultural refuse, carcass formation, and household industrial waste.

**Community Participation**

The competent local government entity tasked with lake maintenance is well-known to the community. Locals are unaware of any government policies or initiatives pertaining to the lake's restoration or conservation. Local governments and non-governmental organisations had never before organised an awareness campaign to encourage the public to protect and sustain the lake and its environs. Locals are interested in contributing to the lake's maintenance, but they are unable to do so without formal assistance.

**Recreational /Tourism Activities**

The lake's vicinity is devoid of consistent or sporadic recreational or tourism activities due to the pollution that permeates the area. Occasional tourism and recreational activities around the lake are favoured by the local populace, provided that the lake is maintained by the local government.

**Institutional Role**

The maintenance and cleansing responsibilities have been neglected for over two decades by the governing body. There is no policy implementation on the lake. The area lacks a formal encroachment inspection. There are no socioeconomic, cultural, recreational, or tourism-related activities occurring in the vicinity of the lake. There has never been an awareness campaign regarding the lake's protection. The KakoriTown undertakes the task of cleaning the lake's periphery every two to three years.

**Sagar Lake**

The lake is situated within ward no. 7 and is enclosed by ward no. 3 and ward no. 11. Sagar Lake, with an expansive expanse of 8.66 acres, ranks as the second largest lake within the KakoriTown.

**Land Use and Population Density**

Residential areas and vacant land border the lake on the south and north, respectively, while agricultural areas border the lake on the east and west. In comparison to the LMC population density of 85PPH in 2011, Ward No. 7 is nearly 1.4 times (118PPH) denser, Ward No. 3 is 1.5 times (126PPH), and Ward No. 11 is 2 times (168PPH).

**Encroachment**

A decade of reduction resulted in the lake's total area decreasing from 9.51 acres in 2011 to 8.66 acres in 2023, a decrease of 0.85 acres. The built-up area surrounding the aquatic body, with a 10-meter buffer zone, is 0.33 acres. Open space is 2.15 acres on the periphery (10-meter buffer zone around the body of water). The ratio of built-up to open space in the 10m buffer zone surrounding the body of water is 1:6.5. 8.16 acres of built-up area is located in the influence area, which is 100 metres from the lake's shore. 33.2 acres are designated as open space in the lake's influence area, which is 100 metres from the lake's shore. The ratio of built-up to non-built-up areas within the lake's influence area, measured 100 metres from the lake's shoreline, is 1:4.07.

**Community Use**

Due to the substantial sewage discharge into the lake, which worsens the water quality, the lake's seasonal commercial fishing is the only community use that is preferred by the residents. This lake has been granted on lease in two portions. Seasonal commercial fishing is conducted for five to six months annually. Animal grazing occurs frequently in the vicinity of the lake's periphery. The remaining activities, including personal laundry, angling, irrigation, animal bathing, earthen ware baking, and other religious practices, are not being observed.

**Pollution**

The pollution in the vicinity of the lake has a detrimental effect on the health of the lake's inhabitants and has also degraded the water quality. Untreated domestic sewage discharge, scat, and municipal solid refuse dumping along the lake's shoreline are the primary sources of pollution. Specific categories of waste, such as agricultural residue, religious or cultural refuse immersion, carcass formation, and household industry waste dumping along the lakeshore, are not being detected.

**Community Participation**

The local authority responsible for lake maintenance is recognized by the community. However, residents are unaware of any policies or initiatives by the government for the lake's restoration or conservation. There has been no previous effort by local governments or NGOs to raise public awareness about protecting and sustaining the lake and its surroundings. Although locals are willing to help maintain the lake, they need official support to do so.

**Recreational /Tourism Activities**

There are no regular or occasional recreational/ tourism activities around the lake, because of the pollution in and around the lake. Occasional tourism and recreational activities around the lake are favoured by the local populace, provided that the lake is maintained by the local government.

**Institutional Role**

The lake is cleaned by the lessee once every two to three years. The local authority has not performed any cleansing or maintenance in over three decades. Failure to enforce policies regarding the lake, there is an absence of formal encroachment checks in the vicinity, and mining operations do not occur around the lake's perimeter. There are no socioeconomic or cultural activities, recreational or tourism-related activities, or awareness campaigns taking place in the vicinity of the lake. Moreover, there has never been an effort to preserve the lake. The KakoriTown undertakes the task of cleaning the lake's periphery every three to four years.

**Chaudhary Mulaka Lake**

The lake is situated in the third ward and is enclosed by the seventh and fourth wards. The lake as a whole has been leased for the past 25 to 30 years. Chaudhary Mulaka Lake encompasses a total area of 1.86 acres.

**Land Use and Population Density**

Residential areas, vacant land, and agricultural land encircle the lake. Ward number three is 1.5 times (126PPH) denser than the LMC population density of 85PPH in 2011. Ward number seven is nearly 1.4 times (118PPH) denser, while ward number four is 2.4 times (194PPH) denser.

**Encroachment**

With a reduction of 0.47 acres over a decade, the lake's total area shrunk from 2.57 acres in 2011 to 2.1 acres in 2023. The constructed area surrounding the water body, with a 10-meter buffer zone, is 0.53 acres. Open space is 1.28 acres on the periphery (10-meter buffer zone around the body of water). The ratio of built-up to open space in the 10m buffer zone surrounding the body of water is 1:2.41. At a distance of 100 metres from the lake's shoreline, the developed region encompasses 6.52 acres. The extent of open space within the lake's influence area, which is 100 metres from the lake's shoreline, is 12.36 acres. The ratio of built-up to non-built-up areas within the lake's influence area (100 metres from the lake's shoreline) is 1:1.89.

**Community Use**

Local residents prefer only seasonal commercial fishing and summertime baking of earthen wares on the lake, as the lake's water quality deteriorates due to the heavy effluent discharge into it. Five to six months per year, seasonal commercial fishing is conducted. Irrigation, personal laundry, animal bathing, grazing along the lake's periphery, and religious and cultural practices such as immersion in idols, cremation ceremonies, and Mundan ceremonies are not observed by the community.

**Pollution**

Untreated Domestic sewage discharge in the lake, Scat, Municipal Solid waste dumping within or in shoreline of lake are regular waste in-or around the lake. The non-regular waste types are Agricultural Residue, Immersion of Religious or Cultural waste, Carcass formation, Household industry waste dumping within or in shoreline of lake.

**Community Participation**

The accountable local government body tasked with lake maintenance is well-known to the neighbours. Concerning the restoration and conservation of the lake, locals are uninformed of government initiatives and policies. No local governmental organisation or non-governmental organisation had ever conducted an awareness campaign to educate the public about the need to preserve the lake and its environs. The local populace, while enthusiastic about participating in lake maintenance, is unable to do so without official assistance.

**Recreational /Tourism Activities**

The lake's vicinity is devoid of consistent or sporadic recreational or tourism activities due to the pollution that permeates the area. Occasional tourism and recreational activities around the lake are favoured by the local populace, provided that the lake is maintained by the local government.

**Institutional Role**

Once in 2-3 years KakoriTown takes the effort to clean the periphery of the lake. However, no proper cleaning and maintenance has been ever done from more than 20 years by the authority. No Implementation of policies on lake. The area lacks a formal encroachment inspection. No Socio-economic or socio-cultural activities are happening around the lake. No recreational and tourism activities are happening around the lake. There has never been an awareness campaign regarding the lake's protection. At last, only the lease holder of the lake cleans the lake in 2-3 years through the process of chlorination.

**Kunwar Gatta Lake**

Two districts encircle the lake, specifically Ward No. 3 and Ward No. 4. The lake in its entirety is leased for a period exceeding thirty years. The lake encompasses a total area of 2.1 acres

**Land Use and Population Density**

Agricultural land and residential areas encircle the lake. Ward no. 3 is 1.5 times (126 PPH) denser than the LMC population density (85 PPH) in 2011, whereas Ward no. 4 is 2.4 times (194 PPH) denser.

**Encroachment**

The lake's overall area experienced a decline from 1.56 acres in 2011 to 1.29 acres in 2023, signifying a reduction of 0.27 acres over the course of a decade. 0.59 acres are developed along the perimeter (10-meter buffer zone of the body of water). The perimeter open space (10-meter buffer zone around the body of water) is 0.66 acres. The ratio of built-up to open space in the 10m buffer zone surrounding the body of water is 1:1.12. 3.29 acres of built-up area is located in the influence area, which is 100 metres from the lake's shore. 8.74 acres are designated as open space in the lake's influence area, which is 100 metres from the lake's shore. The ratio of built-up to non-built-up areas within the lake's influence area (100 metres from the lake's shoreline) is 1:2.65.

**Community Use**

The poor water quality caused by the excessive sewage discharge into the lake discourages any community use of the lake during the summer, with the exception of commercial fishing and baking earthen wares. Seasonal commercial fishing is conducted for five to six months annually. Additionally, grazing is employed on the lake's periphery. Activities such as personal hygiene, fishing, irrigating, bathing animals, baking earthen wares, and engaging in religious or cultural practices like cremation, mundan ceremonies, chat pujas, and idol immersions.

**Pollution**

Significant contributors to lake pollution include the discharge of untreated domestic sewage, municipal solid refuse dumping within or along the lake's shoreline, and SCAT. The pollution in the vicinity of the lake has a detrimental effect on the well-being of the lake's inhabitants and has exacerbated the condition of the water. Agricultural residue, religious or cultural refuse immersion, carcass formation, and household industry waste dumping along the lake's shoreline are not identified as sources of pollution.

**Community Participation**

The entity responsible for lake maintenance is well-known within the community. Despite this, locals are not informed about any government efforts or programs aimed at restoring or conserving the lake. There have been no awareness campaigns organized by local governments or NGOs to encourage public involvement in protecting the lake and its surroundings. The community is interested in contributing to the lake's upkeep but needs formal support to do so.

**Recreational /Tourism Activities**

Occasional or regular tourism or recreational activities are prohibited in the lake's vicinity due to the pollution that permeates the area. Occasional tourism and recreational activities around the lake are supported by the local populace, provided that the lake is maintained by the local government.

**Institutional Role**

There have been no maintenance activities conducted for over three decades. There is no policy implementation on the lake. There is no mechanism in place to prevent encroachment in the vicinity. There are no socioeconomic or cultural activities occurring in the vicinity of the lake. There are no tourism or recreational activities occurring in the vicinity of the lake. There has never been an awareness campaign regarding the lake's protection. The lagoon has never been cleaned by a local government agency. The Kakori Nagar Panchayat undertakes the task of cleaning the lake's periphery every three to four years. The lease holder is obligated to remove all contaminants from the lake every two to three years via chlorination.

**Hauda Lake**

The lake is enclosed within three districts, namely districts 1, 6, and 9. Hauda lake, the largest in Kakori, has been enlarging its perimeter for the past decade. The lake level has risen as a result of the discharge of untreated sewage water from the adjacent residential areas and the consumption of agricultural land situated in its vicinity due to the low-lying terrain.

**Land Use and Population Density**

The population densities of Ward No. 1 (nearly threefold, 269.PPH), Ward No. 6 (twofold, 179 PPH), and Ward No. 9 (1.8 times, 153 PPH) exceed the LMC population density of 85PPH in 2011.

**Encroachment**

The lake experienced a notable expansion from 9.68 acres in 2011 to 15.6 acres in 2023. A discrepancy of 5.92 acres exists. The built-up area surrounding the water body, with a 10-meter buffer zone, is 0.16 acres. A perimeter open space area of 17.66 acres (10-meter buffer zone around the body of water). The ratio of built-up to open space in the 10m buffer zone surrounding the body of water is 1:110.37. Influence area (located 100 metres from the lake's shoreline) developed land comprises 18.56 acres. Open space comprises 53.72 acres in the lake's influence area, which is located 100 metres from the lake's shore. The ratio of built-up to non-built-up areas within the lake's influence area (100 metres from the lake's shoreline) is 1:2.89.

**Community Use**

This lake's proprietorship is delineated into four portions. Seasonal commercial fishing is conducted for five to six months per year, and dairy production occurs consistently. Due to the substantial sewage discharge into the lake, which worsens the water quality, the lake's seasonal commercial fishing is the only community use that is preferred by the residents. Grazing by animals is observed in the vicinity of the lake. Aspects such as personal hygiene practices, fishing, irrigation, animal bathing, earthenware baking, and religious and cultural activities (e.g., idol immersion, mundan ceremony, chat puja), are not observable within or in the vicinity of the lake.

**Pollution**

Significant pollutant sources include untreated domestic sewage discharge, municipal solid refuse dumping within or along the lake's shoreline, and scat. Non-obtrusive sources of pollution in the vicinity of Hauda Lake include agricultural residue, cultural or religious refuse immersion, carcass formation, and the dumping of household industrial waste along the lake's shoreline. The pollution in and around the lake has a detrimental effect on the health of the lake's inhabitants and has deteriorated the water quality.

**Community Participation**

The community is aware of the local government body in charge of maintaining the lake. Nonetheless, there is a lack of awareness among residents regarding government policies or initiatives for the lake's restoration and conservation. Neither local governments nor NGOs have conducted awareness campaigns to promote the protection and sustainability of the lake and its environment. While locals are keen to participate in the lake's maintenance, they require formal assistance to proceed.

**Recreational /Tourism Activities**

Occasional or regular tourism or recreational activities are prohibited in the lake's vicinity due to the pollution that permeates the area. Locals are in the favour of the occasional recreational/ tourism activities around the lake if the lake is maintained by the local govt. body.

**Institutional Role**

There have been no maintenance activities conducted for over three decades. The chlorination process is exclusively employed by the lease proprietor of the lake every two to three years. The Kakori Nagar Panchayat endeavours to conduct perimeter cleaning of the lake every one to two years. There is no policy implementation on the lake. There are no routine encroachment checks in the area. There are no socioeconomic or cultural activities occurring in the vicinity of the lake. There are no tourism or recreational activities occurring in the vicinity of the lake. There has never been an awareness campaign regarding the lake's protection. The lagoon has never before been cleaned by a local government agency.

## FINDINGS

Urbanisation in Kakori has undeniably contributed to the depletion of static water bodies, primarily as a result of pollution and institutional negligence (as ranked by user perception in Table 5). As a consequence, the environment, water body boundaries, and the overall quality of life in urban areas have been profoundly affected.

**The above-mentioned parameters can be summarised as follows**:

1. Pollution: Prominent sources of pollution encompass untreated domestic sewage discharge, disposal of municipal solid waste along the lake's shoreline, and scat.These activities have resulted in water contamination that is unfit for human consumption and other applications. The consequence of this situation is a reduction in the accessibility of potable water for the populace.
2. Institutional Role: Due to the indifference and lack of consciousness on the part of their responsibilities of respective local governing bodies, the lakes are susceptible to environmental degradation.
3. Community Participation: The lake and its environs have been subject to a lack of awareness community engagement, and government assistance, resulting in a lack of interest and support to maintain the lakes.
4. Encroachment: The lakes' surface area has fallen drastically due to unauthorised construction and domestic industrial use.
5. Land Use and Population Density: The lakes' susceptibility to degradation is primarily attributable to the district's high population density and the heterogeneous land use character surrounding the lakes.
6. Community Use: The aforementioned factors will prevent the local community from making a substantial and justifiable use of the lake, consequently resulting in inadequate maintenance.
7. Recreational and Tourism Activities: Given the deplorable state of the Kakori lakes, their utilisation for recreational and tourism purposes is implausible. Restoring the lakes and generating economic revenue from them could be the primary objective in this regard.

To address the issue of static water body depletion in Kakori, it is essential to prioritize the conservation of surface water bodies and implement effective management strategies. This involves the efficient administration of existing aquatic environments through public engagement and the enforcement of stringent regulations to prevent further degradation. Additionally, investments in renewable water resources, the promotion of sustainable urban planning, and the enhancement of waste management systems are crucial in mitigating the adverse effects of urbanization on static water bodies.

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**Table 1 Population of Lucknow from 1991-2031***

| Year | Lucknow UA (A) | | Lucknow Planning Boundary (B) | | Lucknow Municipal Corporation | | Lucknow Planning Boundary Excluding LMC* | |
|---|---|---|---|---|---|---|---|---|
| | Population | Decadal Growth Rate* | Population | Decadal Growth Rate* | Population | Decadal Growth Rate* | Population | Decadal Growth Rate |
| 1991 | 1669204 | 65.66 | | | 1619116 | 70.79 | | |
| 2001 | 2245509 | 34.53 | 27,73,618 | - | 21,85,297 | 35.00 | 5,88,321 | |
| 2011 | 2880108 | 28.26 | 35,48,732 | 27.94% | 28,17,105 | 28.87 | 7,31,627 | 24.35% |
| 2021 | 3713003 | 28.92 | 45,50,326 | 28.22% | 36,15,909 | 28.36 | 9,34,417 | 27.71% |
| 2031 | 4787488 | 28.94 | 65,00,000 | 42.84% | 4467158 | 23.54 | 20,32,842 | 117.55% |

Source- Census Tables and Author's Projection *Projected Data for 2021 and 2031

**Table 2 Status of lakes or ponds bodies in Lucknow**

| Year | Lakes/ Ponds within the Boundary of 2031 | | | Lakes/ Ponds within LMC Boundary | | | Lakes/ Pond in Peri-Urban Area | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total No. | Area (sq.km.) | % of Total Area increased/ decreased | Total No. | Area (sq.km.) | % of Total Area increased/ decreased | Total No. | Area (sq.km.) | % of Total Area Increased/ Decreased |
| 2011 | 86 | 0.82 | | 20 | 0.28 | | 66 | 0.54 | |
| 2023 | 78 | 0.74 | -6.1% | 19 | 0.26 | -7.15% | 59 | 0.48 | -11.12% |

Source: Author (Interpreted from satellite image of 2011, 2023)

Note: The mentioned above information excludes the number of wetlands identified by National Water Informatic Centre, Department of Water Resources, River Development and Ganga Rejuvenation, Ministry of Jal Shakti (Govt. of India)

**Table 3 Population Density comparison between Kakori town and Lucknow Municipal Area**

| Town Area | Density (1991) | Density (2001) | Density (2011) |
|---|---|---|---|
| Kakori Nagar Panchayat | 123 PPH | 153 PPH | 176 PPH |
| Lucknow Municipal Area | 49 PPH | 67 PPH | 85 PPH |

Source: Lucknow Master Plan 2031, Census of India (1991, 2001, 2011)

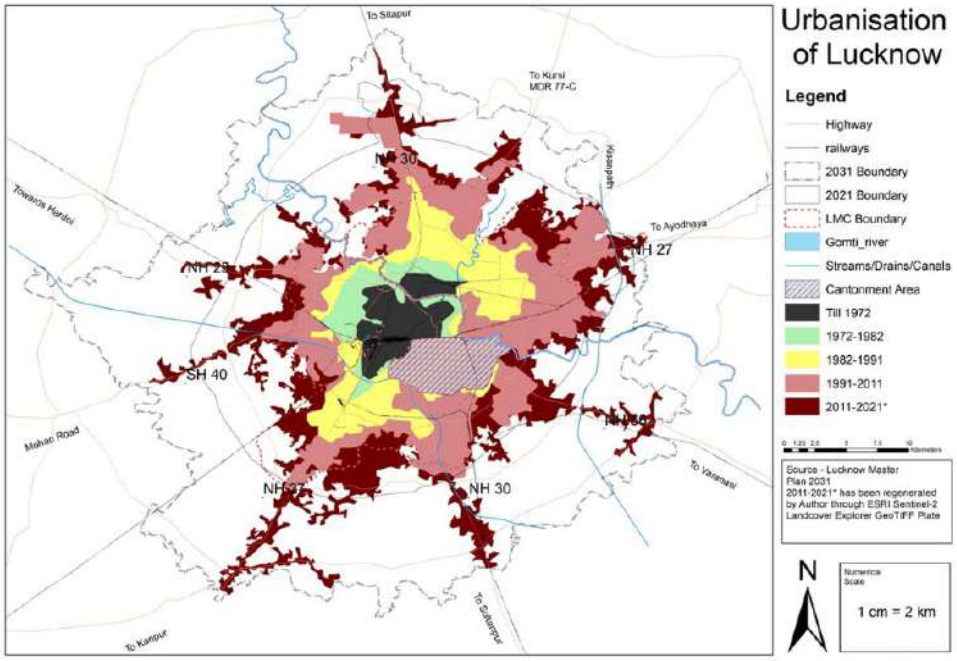
**Table 4 GIS based Surface Area calculation of Static Water Bodies in Kakori for 2011 and 2023**

| S.No. | Identified Lake in Kakori Town | Proximity of lake to urban settlement | Lake area (2011) | Lake area (2023) |
|---|---|---|---|---|
| 1. | Salariya Lake | 2.26 m | 2.57acres | 2.10 acres |
| 2. | Sagar Lake | 6.21 m | 9.51acres | 8.66acres |
| 3. | Chaudhary Mulaka Lake | 1.83 m | 2.22acres | 1.86 acres |
| 4. | Kunwar Gatta Lake | 3.15 m | 1.56acres | 1.29 acres |
| 5. | Hauda Lake | 1.19 m | 9.68acre | 15.6acres |

**Table 5: Parameter vulnerability rank of the all identified lakes based on user perception scoring. (1 - Major concern and 7 - least concern)**

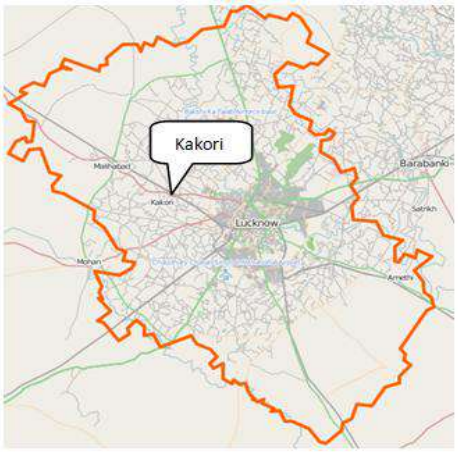
| Parameter | Hauda Lake | Sagar Lake | Kunwar Gatta Lake | Chaudhary Mulaka Lake | Salariya Lake |
|---|---|---|---|---|---|
| Land Use and Population Density | 5 | 5 | 4 | 5 | 4 |
| Encroachment | 4 | 4 | 5 | 3 | 5 |
| Community Use | 6 | 7 | 6 | 6 | 7 |
| Pollution | 1 | 1 | 1 | 1 | 2 |
| Community Participation | 3 | 2 | 2 | 4 | 3 |
| Recreational / Tourism Activities | 7 | 6 | 7 | 7 | 6 |
| Institutional Role | 2 | 3 | 3 | 2 | 1 |

Source: Lucknow Master Plan 2031 (2011-2021 has been regenerated by Author through ESRI Sentinel-2Landcover Explorer GeoTIFF Plate).



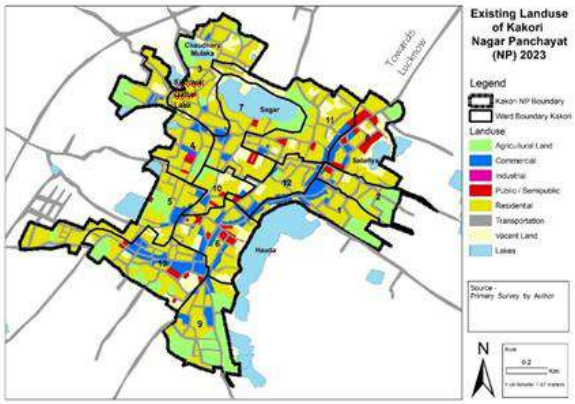

**Source - Re-created by Author**

**Map8 Map showing Urbanisation growth pattern of Lucknow**



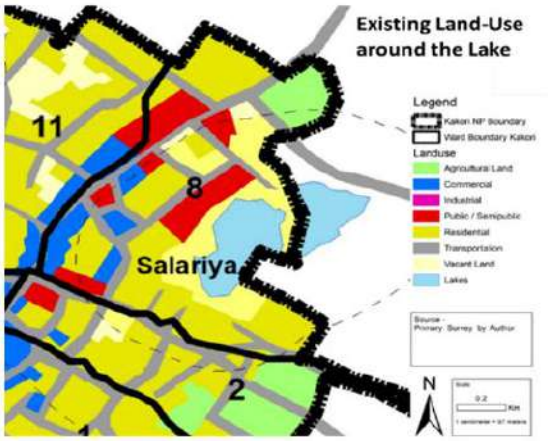

**Source: Open Street Map.com**

**Map 2 Map showing Location of Kakori Town in Lucknow District**



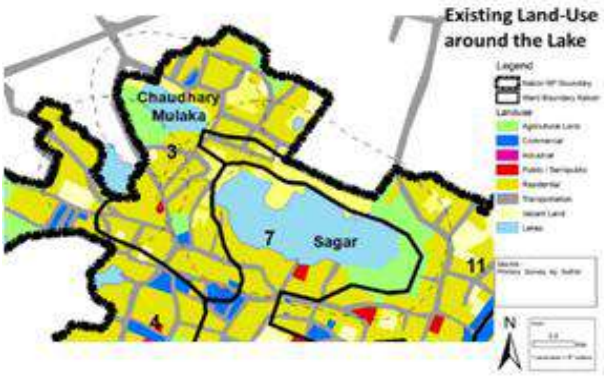

**Source - Author**

**Map3 Existing Landuse Map of KakoriTown 2023**



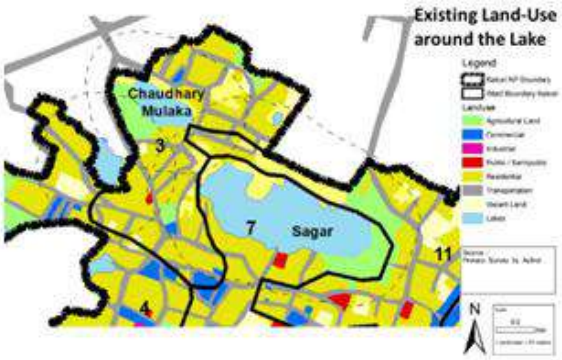

**Source- Author**

**Map 4 Landuse character around Salariya Lake**



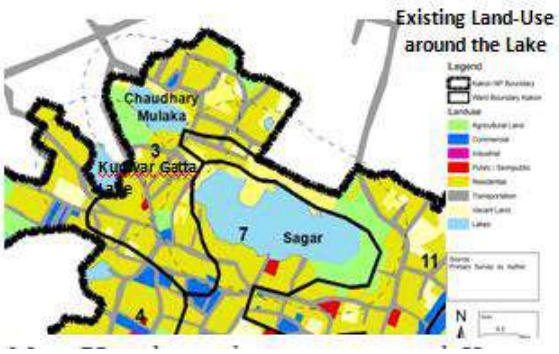

Source- Author

**Map 5 Landuse character around Sagar Lake**



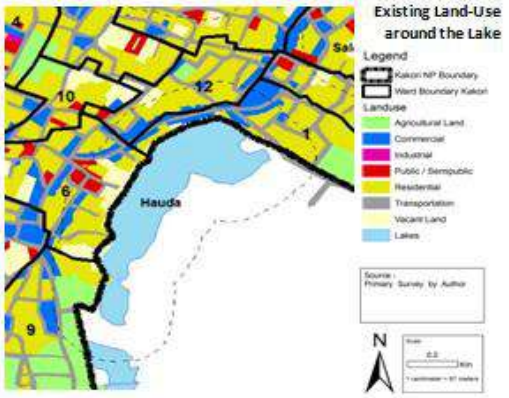

Source- Author

**Map 6 Landuse character around Chaudhary Mulaka Lake**



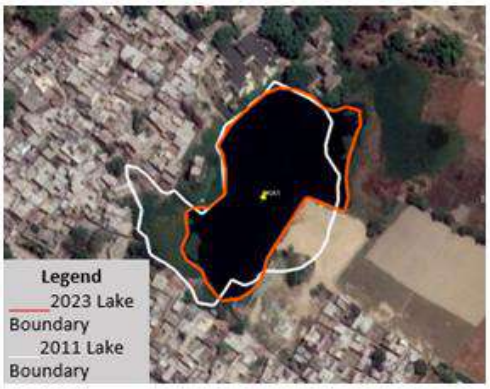

Source- Author

**Map 7 Landuse character around Kunwar Gatta Lake**




**Source - Author**

**Map 8 Landuse character around Hauda Lake**




**Source- Satellite Imagery**

**Image 1 Change in surface area of Salariya lake**

Source- Author

**Image 2 Image showing community use of Salariya lake**

Source- Author

**Image 3 Image showing pollution around Salariya lake**

Source- Satellite Imagery

**Image 4 Change in surface area of Sagar Lake**

Source- Author

**Image 5 Image showing Animal grazing around the Sagar Lake**

Source- Author

**Image 6 Image showing Pollution and Encroachment around the Sagar Lake**

Source- Satellite Imagery

**Image 7 Change in surface area of Chaudhary Mulakalake**

Source- Author

**Image 8 Image showing community use of the lake**

**Source- Author**

**Image 9 Image showing pollution around the lake**

**Source- Satellite Imagery**

**Image 10 Change in surface area of Kunwar Gatta lake,**

Source - Author

**Image 11 Image showing community use of the lake**

Source: Satellite Image of 2011, 2023

Source- Satellite Imagery

**Image 12 Change in surface area of Hauda lake**

**Image13 Image Showing Commercial Fishing in Hauda Lake**

**Image14 Image Showing Pollution Around the Hauda Lake**

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**RESEARCH ARTICLE**

# A Thematic Exploration of Insights and Reflections on E-Governance Adoption and Challenges in Anantnag, Jammu and Kashmir

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## ABSTRACT

The paper rigorously examines the seismic shift from traditional Governance structures to the dynamic landscape of e-Government. It meticulously dissects how e- Governance serves as a catalyst in upholding the fundamental tenets of good Governance , specifically emphasizing citizen participation, elevating service delivery standards, and fostering unparalleled transparency within administrative frameworks. The study is designed to intricately unravel the intricate web of agencies and diverse stakeholders entrenched in the e-Governance ecosystem. It aims to spotlight their distinct roles and unequivocal contributions towards the effective orchestration of e- Governance mechanisms. Moreover, the research fervently scrutinizes the multifaceted interactions intrinsic to e-Governance , examine into pivotal realms like Government-to-citizen (G2C) and other transformative dimensions. It critically analyses the vast array of tools and resources accessible to citizens and government bodies, empowering their seamless participation and engagement in the e- Governance paradigm. The study embarks on a relentless examination, conducting a rigorous SWOT analysis, specifically targeting the e- Governance landscape in the district of Anantnag in Jammu and Kashmir. It aims to unearth the strengths, weaknesses, opportunities, and threats inherent in the local e- Governance framework. In addition, the research meticulously delineates the extensive gamut of services rendered accessible through e- Governance platforms, vividly portraying the breadth and depth of online provisions available to citizens, propelling the region towards an era of enhanced accessibility and streamlined service delivery. The paper examines the challenges entrenched within e- Governance implementation. It presents an assertive array of suggestions designed to galvanize substantial improvements in the e- Governance landscape, fostering an environment conducive to heightened efficiency and inclusivity.

**Keywords:** Governance , Services, Stakeholders, Strength, lacunas.

# INTRODUCTION

Electronic Governance (or e-Gov) refers to the use of information and communication technologies (ICTs) by governments in (Relyea, H. C. 2002) delivering public services, exchanging information, communicating with citizens, businesses, and other government entities, and conducting Governance -related activities. It involves the digitization of government operations and the integration (Gronlund, Å., & Horan, T. A. 2005) of technology into various administrative processes to improve efficiency, transparency, accessibility, and accountability in the delivery of public services. Electronic Governance encompasses a wide range of initiatives and applications, including online portals for accessing government services, digital databases (Fath-Allah et., al 2014) for storing and managing information, electronic voting systems, online tax filing, digital identity systems, and more. Its primary goal is to leverage technology to transform Governance , making it more responsive, efficient, and citizen-centric. The adoption of e- Governance by administrations is driven by various compelling factors. Firstly, it aims to enhance (Hujran, O, et., al 2023) efficiency and effectiveness in delivering public services. Through digital platforms, governments can streamline processes, reduce bureaucratic hurdles, and offer faster and more accessible services to citizens. e-Governance facilitates transparency and accountability. By digitizing processes, administrations (Hirwade, M. A. 2010) can maintain clearer records, enabling better tracking of government actions and expenditures. The transparency helps in curbing corruption and building trust between the government and its citizens.

Electronic Governance also promotes (Sakolkar, P. C. 2023) citizen engagement and participation in Governance processes. Digital platforms enable easier access to information, allowing citizens to interact with the government, voice concerns, provide feedback, and participate in decision-making, thereby fostering a (Malodia, S., 2021) more inclusive Governance system. Adopting e- Governance aligns with the broader global trend of technological advancement. Governments aim to keep pace with technological developments to harness their benefits and stay relevant in an increasingly digital world. Lastly, for regions like Anantnag, Jammu and Kashmir, where geographical (Manhas, J., & Mansotra, V. 2011) challenges might hinder traditional Governance methods, e- Governance provides a means to bridge these gaps and reach remote or less accessible areas with essential services and information.

**Theoretical framework**

The theoretical framework of the study on Adoption and Challenges of e-Governance in Anantnag, Jammu and Kashmir revolves around understanding the intricate interplay between technology adoption, administrative processes, and societal implications. Drawing from models of technological adoption, such as the (Davis, F. D. 1989) Technology Acceptance Model (TAM) or the Diffusion of Innovations theory, the research aims to explore the factors influencing the (Dearing, J. W., & Cox, J. G. 2018) acceptance and utilization of e-Governance tools among different stakeholders. It considers institutional theories to analyse the role of government structures, policies, and institutional arrangements in facilitating or hindering the adoption of digital Governance practices. Societal perspectives, including social inclusion, access to technology, and digital literacy, are also central to comprehending the challenges faced in implementing e-Governance initiatives within the specific socio-cultural context of Anantnag District. The framework seeks to provide a holistic understanding by integrating technological, institutional, and societal dimensions to elucidate the complexities surrounding e-Governance adoption and its challenges in the region.

**Significance of the Study**

The study holds significance due to its comprehensive examination of the transition from traditional Governance to e-Government in Anantnag, Jammu and Kashmir. It sheds light on how e-Governance promotes good Governance principles, enhances citizen participation, and improves service delivery while addressing challenges and proposing solutions for a more efficient and inclusive Governance landscape.

**Objectives of the study**

The main objectives of the study are to comprehensively explore the transition from traditional Governance to e-Government, highlighting its role in upholding good Governance principles with a focus on citizen participation, service delivery enhancement, and transparency. The study aims to dissect the intricate network of stakeholders in the e-Governance ecosystem, emphasizing their roles in orchestrating effective mechanisms, while scrutinizing the interactions within Government-to-citizen (G2C) dimensions. It seeks to critically analyze the available tools for citizen and government engagement, conducting a SWOT analysis specific to the e-Governance landscape in Anantnag, Jammu and Kashmir, to uncover inherent strengths, weaknesses, opportunities, and threats. Moreover, the research aims to delineate the breadth and depth of e-Governance services offered, aiming for improved accessibility and streamlined delivery, while also identifying and proposing solutions to the challenges entrenched in e-Governance implementation for fostering an environment of efficiency and inclusivity.

## MATERIAL AND METHODS

The study adopted a comprehensive mixed-methods approach, combining document analysis and Google Forms to gather primary and secondary data thoroughly. With a qualitative focus, it aimed to deeply understand the Adoption and Challenges of e-Governance in Anantnag District, Jammu and Kashmir. Structured interviews involved 15 government personnel, web developers, Khidmat Center and Common Service Center personnel's, and National Informatics Centre personnel. Additionally, open-ended questions extracted insights from these officials, unveiling significant themes and patterns. The study enriched its depth and context by incorporating secondary data from various sources such as books, journals, articles, government documents, and web resources.

## RESULT AND DISCUSSION

**Shift from traditional Governance to e- Governance**

The shift from traditional Governance to e- Governance represents a fundamental transformation in how governments (Saxena, K. B. C. 2005) operate and interact with their constituents. The evolution has been driven by several compelling factors. The integration of information and communication technologies (ICTs) has revolutionized the way governments function. Traditional Governance often relied on manual, paper-based processes, which were time-consuming, prone to errors, and less efficient. e- Governance introduces digital solutions (Seifert, J., & Petersen, R. E. 2002) that streamline administrative tasks, automate processes, and significantly enhance operational efficiency. By leveraging technology, governments can expedite service delivery, reduce bureaucratic hurdles, and optimize resource utilization.

e- Governance embodies the principles of transparency and accountability. The traditional Governance model often faced (Atiq, E., Salim, M., & Mahmood, N. 2023) challenges in maintaining comprehensive records, tracking government actions, and ensuring transparency in decision-making processes. With e-Governance , digital platforms enable better documentation, storage, and accessibility of information, fostering greater transparency. Citizens can access government data, monitor expenditure, and hold authorities accountable, thereby strengthening(Harahap, M. A, et., al 2023) trust in the Governance system.e- Governance fosters citizen engagement and empowerment. Traditional Governance frameworks typically had limited avenues for citizen participation in decision-making processes. However, e- Governance facilitates direct interaction between citizens and government entities (Singh, A. 2023) through online portals, social media platforms, and other digital channels. This increased accessibility enables citizens to voice their concerns, provide feedback, participate in policy formulation, and access government services more conveniently. The global technological revolution has played a pivotal role in the shift towards e-Governance . With the rapid advancement of digital technologies, governments worldwide recognize the need to adapt and harness these innovations to modernize Governance practices. Embracing e- Governance allows (Sharma, P. 2023) administrations to keep pace with technological developments, remain relevant, and harness the benefits of digital transformation. The transition from traditional Governance to e- Governance signifies a paradigm shift towards

more efficient, transparent, and citizen-centric Governance models that harness the potential of technology to better serve the needs of society.

**Stakeholders and Agencies in Electronic Governance Implementation**

The government of Jammu and Kashmir has undertaken several significant initiatives aimed at leveraging technology to enhance (Dar, S. A. (2022) Governance and public service delivery. One of the notable initiatives is JAKeGA, an online grievance redressal system, which provides citizens a platform to register complaints and seek resolution from relevant authorities. The digital platform streamlines the grievance redressal process, ensuring a more efficient and transparent mechanism for addressing citizen concerns. The establishment of Common Service Centres (CSCs) and Khidmat Centres across (Sharma, A., & Mir, A. Q. 2022) the region plays a pivotal role in providing various government services to citizens at their doorstep. These centres serve as access (Dar, S. A. 2022) points for a wide range of services, including application submissions, certificates, payments, and information dissemination. They bridge the gap between citizens and government agencies, making services more accessible and convenient for the public.

The creation of Software Technology Parks reflects the government's commitment to fostering a conducive environment for technological advancements and innovation. These parks serve as hubs for IT companies, start-ups, and entrepreneurs, promoting the growth of the IT sector in the region and (Dar, S. A., & Sakthivel, P. 2021) creating opportunities for employment and economic development.Aligning with the National e- Governance Plan (NeGP), the government has collaborated with web developers and the National Informatics Centre (NIC) to enhance digital infrastructure and develop user-friendly web portals and applications. These initiatives aim to improve access to information and government services while ensuring a seamless and user-centric online experience for citizens. The establishment of National Informatics Centre offices in each (Sharma, P. (2023) district signifies a decentralized approach to technology implementation. These centres facilitate the integration of digital technology into various administrative processes at the local level, ensuring that the benefits of e- Governance reach every corner of the region, addressing specific district-level needs and challenges.

Electronic Governance aims to transform Khush Hal State into a realm of genuine prosperity and well-being. By focusing on efficient Governance , transparency, and citizen engagement, these initiatives strive to create a (Bhatnagar, S. 2003) state where technology serves as a catalyst for progress. Through digitalization, the goal is to enhance accessibility to services, foster economic growth, and empower every individual, ensuring an inclusive and prosperous society for all. These initiatives collectively represent the government's concerted efforts to harness technology for (Dar, S. A. 2023) efficient Governance , citizen empowerment, and socio-economic development in Jammu and Kashmir. Through these measures, the government endeavours to create a more inclusive, responsive, and digitally-enabled Governance framework that caters to the diverse needs of its populace.

**Electronic Governance : Paving the Way for Good Governance**

The adoption of e- Governance is deeply rooted in the pursuit of achieving good Governance by revolutionizing traditional (Telino, V., et., al 2020) administrative processes. It aims to enhance Governance practices in various ways, primarily by ensuring efficiency, transparency, accountability, and citizen-centricity.Efficiency in Governance is greatly improved through e-Governance . For instance, online portals for government services streamline processes, reducing the time taken for tasks like applying for permits or licenses. The efficiency is evident in systems like digital tax filing, where citizens can submit returns swiftly, reducing both administrative burdens (Falk, S., Rommele, A., & Silverman, M. 2017) and turnaround time.Transparency is another hallmark of good Governance that e- Governance fosters. Digital platforms enable the publication of government decisions, expenditure details, and policy formulations, ensuring that citizens have access to comprehensive and accurate information. For instance, budgetary allocations and spending records are made publicly available, allowing for scrutiny and accountability.

Accountability is bolstered through e- Governance systems. For instance, grievance redressal mechanisms like online complaint (Lindquist, E. A., & Huse, I. 2017) portals ensure that citizens' concerns are logged and addressed

systematically. Authorities can track the progress of complaints, ensuring accountability in resolving issues raised by citizens. e- Governance emphasizes citizen-centricity by placing services and information within reach of the public. For instance, educational portals provide access to resources, exam results, and scholarships, ensuring equal (Monga, A. 2008) opportunities for education. This approach puts citizens at the center of Governance , making services more accessible and responsive to their needs.e- Governance aligns with the principles of good Governance by enhancing efficiency, promoting transparency, fostering accountability, and prioritizing citizen-centric services. These examples showcase how e- Governance initiatives contribute to creating an environment conducive to good Governance practices.

**Jammu and Kashmir Public Services Guarantee Act of 2011**

The Jammu and Kashmir Public Services Guarantee Act of 2011 is a significant legislative measure designed to empower citizens by ensuring timely delivery of public services. The act outlines a framework that guarantees specific public services within a stipulated time frame to the people of the state. It essentially sets standards for service delivery, making government departments (Singh, H. 2021) accountable for providing services promptly and efficiently.Under this act, eligible individuals have the right to receive public services within predetermined time limits. These services can range from obtaining certificates, licenses, permits, or availing specific government schemes. The act specifies the time duration within which these services must be delivered, thereby holding government officials and departments accountable for meeting these deadlines. The act introduces a mechanism for citizens to seek compensation in case of delays or deficiencies in service delivery. If the specified services are not provided within the mandated time frame or are found to be deficient, eligible individuals have the right to claim compensation. The provision aims to ensure that citizens receive the services they are entitled to without undue delays or shortcomings.

By enacting this law, the government(Kundan, M. S. 2018) aims to instill accountability, transparency, and efficiency in public service delivery. It places the onus on government agencies to prioritize citizens' needs, ensuring that they receive essential services within a reasonable and predefined time frame. The provision for compensation acts as a deterrent against negligence or inefficiency on the part of government officials, thereby enhancing the quality and timeliness of service delivery to the people of Jammu and Kashmir. The Jammu and Kashmir Information Technology Act of 2000 aims to enhance e-services delivery within the region. This act focuses on the (Wani, A. A., & Parray, J. A. 2023) establishment of a legal framework to govern electronic transactions, digital signatures, cyber security, and the use of electronic records, thereby facilitating the delivery of various electronic services to the citizens of Jammu and Kashmir.

**e- Governance service**

Jammu and Kashmir has emerged as a leader in the realm of e- Governance service provision, boasting an impressive array of 1028 online services, as reported by the National e- Governance Division (NeSDA). The region's remarkable achievement in delivering electronic services reflects its dedication to (Dar, S. A. 2022) embracing digital solutions for Governance . According to the NeSDA report, J&K stands out prominently for its extensive online service portfolio, underscoring its commitment to enhancing accessibility and efficiency for citizens. This accomplishment highlights the state's proactive approach in leveraging technology to cater to a diverse range of public needs. The proliferation of online services signifies a significant stride (Economic Times NeSDA report 2023) towards a more inclusive and digitally empowered Governance framework in Jammu and Kashmir. This success story positions the region as a frontrunner in embracing e-Governance , marking a significant milestone in its journey towards leveraging technology for the betterment of its citizens.

**SWOT of Electronic Governance**

The strengths lie in the advancements made in increased connectivity, supported by government initiatives and citizen engagement tools. These factors, coupled with a skilled IT workforce, create a solid foundation (Jiang, H. 2023) for technological growth and innovation. However, various weaknesses pose challenges. Infrastructural limitations hinder the seamless operation of technology, while the digital divide and limited internet penetration

create disparities in access, limiting the reach of digital initiatives. Language barriers further impede the widespread adoption and effectiveness of technological solutions. Amid these challenges, opportunities emerge. Efforts directed towards (Chinnasamy, V. 2017) rural outreach initiatives can bridge the gap, leveraging technological advancements for inclusive growth. Continued technological progress offers opportunities for innovation and development, potentially boosting tourism, economic activities, and prompting investments in IT infrastructure.

Yet, threats loom over these opportunities. Cyber security vulnerabilities present risks as connectivity expands, potentially (Dar, S. A., & Sakthivel, P. 2021) compromising systems and data. Resistance to adopting new technology, political instability, and the unpredictability of natural disasters also pose significant threats, potentially disrupting progress and infrastructure. Balancing these strengths, weaknesses, opportunities, and threats becomes crucial in navigating the landscape of technology and connectivity, requiring strategic measures to leverage strengths, address weaknesses, seize opportunities, and mitigate threats for a more resilient and inclusive technological ecosystem.

**Electronic Governance Interactions**

**Government-to-Business (G2B)**

This model denotes interactions between government entities and businesses. It encompasses services, regulations, and collaborations designed to facilitate business operations and stimulate economic growth. For instance, online licensing and permit applications, tax filings, procurement portals, and business registration platforms are examples of G2B interactions. These initiatives streamline processes, reduce bureaucracy, and enhance transparency, making it easier (Panayiotou, N. A., & Stavrou, V. P. 2021) for businesses to interact with the government. The goal is to create an environment conducive to business development and investment, fostering economic prosperity.

**Government-to-Citizen (G2C)**

G2C interactions refer to services and interactions between the government and individual citizens. This includes various online platforms and services aimed at providing citizens with easy access to government information, services, and assistance. Examples of G2C interactions are online (Katoch, R. 2016) portals for paying taxes, applying for government benefits, accessing healthcare services, obtaining identification documents, and registering for various government programs. These initiatives focus on convenience, accessibility, and inclusivity, aiming to improve the quality of life for citizens by ensuring efficient access to essential government services.

**Government-to-Government (G2G)**

G2G interactions involve communication, collaboration, and data sharing between different government agencies or departments, either at the same administrative level or across different administrative tiers. These interactions facilitate the exchange of information, resources, and services among government (Palma, J. P. B., et., al 2023)entities to enhance operational efficiency, avoid duplication, and streamline processes. An example is the sharing of data between tax authorities and social services agencies to verify eligibility for government assistance programs. G2G initiatives aim to promote coordination and cooperation among government bodies to better serve the public.

**Government-to-Employee (G2E)**

G2E interactions encompass the communication and services provided by the government to its employees. These interactions involve human resources management, training programs, payroll services, benefits (Rao, V. R. 2011) administration, and internal communications within government organizations. G2E initiatives aim to enhance employee productivity, satisfaction, and efficiency by providing streamlined and accessible services for the workforce. Each of these models represents distinct facets of government interaction, emphasizing efficiency, accessibility, and transparency in service delivery across various sectors, entities, and individuals within society.

**Challenges of electronic Governance in Jammu and Kashmir**

Electronic Governance in Jammu and Kashmir encounters specific regional challenges that impact its implementation. The region's remote and mountainous terrain creates infrastructural limitations, leading to inadequate digital infrastructure and connectivity. The limitation in internet penetration hampers (Dar, S. A. 2022)

the widespread accessibility of online services. Moreover, geographical barriers and varying levels of accessibility in remote areas contribute to disparities in technology access and digital literacy, posing hurdles in uniform service delivery. The region's multilingual population presents challenges in disseminating information uniformly, necessitating the development of platforms accommodating diverse linguistic needs. Apart from regional challenges, Jammu and Kashmir faces special obstacles in the realm of e-Governance . Security concerns stemming from the sensitive geopolitical situation pose significant threats. Cyber security vulnerabilities and potential data breaches heighten risks within e- Governance systems. A significant data breach has affected the data system at Kashmir University, prompting an urgent inquiry from the Computer Emergency Response Team (CERT). Reports indicate that (Kashmir Observer(2022) sensitive information of over one million students, including registration details, email IDs, and passwords, was purportedly available for purchase on a 'Dark Web' forum for $250. The breach has sparked demands from students and teachers for an independent investigation by a MeitY team to establish accountability and fortify the system's security. Allegations suggest that previous warnings about potential data threats within the University went unheeded. While the University authorities claim that the data appears unmodified, they are conducting further analysis. Despite certain data being (M. Karpiuk, J. Kostrubiec 2022) accessible in the public domain, the University intends to take appropriate legal measures based on in-depth analyses of any potential breaches.

Political instability and uncertainties can disrupt the continuity and stability of digital initiatives, affecting ongoing projects (Dar, S. A. (2022) and policies. The region's susceptibility to natural disasters, such as earthquakes and floods, poses risks to digital infrastructure and service continuity during emergencies. The "India Inequality Report 2022: Digital Divide" released by Oxfam India delves into data sourced from the Centre for Monitoring Indian Economy's household survey spanning Jan 2018 to Dec 2021. The report sheds light on glaring gender disparities in digital access, revealing that Indian women are 15 percent less likely to own mobile phones and 33 percent less likely to use mobile internet services compared to men. Shockingly, women constitute just one-third of the country's internet users. Globally, India stands at the forefront of the Asia-Pacific region but with a staggering 40.4 per cent gender gap, marking the widest difference. The report also uncovers a stark rural-urban digital divide, despite a commendable 13 per cent growth in digital adoption, merely 31 percent of the rural population utilizes the internet in contrast to 67 per cent of urban residents. It highlights caste-based disparities, noting that ST households exhibit the lowest (Oxfam India 2022) inclination towards formal financial services, followed by SC and OBC households. Access to computers follows a similar pattern, with General and OBC groups having notably higher rates than SC and ST populations, indicating a significant gap. Even across religions, Sikhs exhibit the highest likelihood of computer ownership, trailed by Christians, Hindus, and finally Muslims. In the global e-participation index, a measure encompassing online service provision, connectivity, and human capacity, India ranks 105 out of 193 nations, emphasizing the room for improvement in its e-Government initiatives.

The theme "Digital Generation, Our Generation" aims to address the digital gap, as per the United Nations. Despite the global shift towards online platforms in the post-COVID-19 era encompassing businesses, education, and healthcare, around 22 billion individuals under 25 lack internet access, with a significant majority being girls. Kashmir faces a particularly alarming scenario due to frequent and extended internet shutdowns in the past two years, significantly hampering online education and causing multiple challenges. The entrenched gender norms in societies like India curtail equitable technology access for women and girls, particularly those from economically disadvantaged backgrounds. These norms contribute to lower digital literacy among girls, unfamiliarity with digital tools, and unequal access to electronic devices. The mobile gender gap report highlights a staggering 56% lower likelihood of Indian women using mobile internet compared to men, with only 35% of active users in the country being women.

Frequent internet shutdowns in Kashmir significantly disrupt and impede the progress of e-Governance initiatives in the region. These interruptions hinder the seamless implementation of digital Governance (Gupta, R., & Kumar, K. 2020) tools and services, affecting various aspects such as communication, access to information, online government services, and connectivity for citizens. The lack of consistent and reliable internet connectivity poses

substantial obstacles to the effective functioning of e-Governance , hindering the region's technological advancements and digital outreach efforts. Indeed, the revocation of Article 370 brought about notable changes in the digital landscape of Kashmir. Post this constitutional change, there was an evident shift towards accelerating the digitization process in the region. Efforts were made to boost technological infrastructure and expand digital (Lalwani, S., & Gayner, G. 2020) initiatives to enhance Governance , public services, and connectivity. However, despite these strides, the recurring internet shutdowns have remained a persistent challenge, intermittently hampering the momentum of the digitization process and the smooth functioning of e-Governance in Kashmir.

Overcoming these challenges demands targeted strategies. Improving digital infrastructure and internet connectivity in remote areas, promoting digital literacy, and developing multilingual platforms can bridge regional disparities. Robust cyber security measures and contingency plans are imperative to address security risks and ensure service continuity. Navigating political transitions and establishing resilient systems capable of withstanding natural disasters are critical for sustaining and advancing e- Governance in Jammu and Kashmir. Collaborative efforts involving government bodies, technology experts, and local communities are pivotal in addressing these challenges and harnessing the full potential of e- Governance in the region.

**Implications of the Study**

The study's social implications are profound, signaling both challenges and opportunities in the realm of e-Governance . Addressing issues like digital literacy gaps among citizens and officials not only ensures effective utilization of government services but also empowers individuals with skills vital for modern participation in Governance . By bridging access gaps for marginalized communities, the study hints at a more inclusive society, where every citizen has equal opportunities to benefit from governmental initiatives. Moreover, the focus on data security and privacy in sensitive regions like Jammu and Kashmir speaks to the larger societal need for trust and protection in the digital age. Additionally, the study underscores the importance of reliable power infrastructure for sustained digital services, highlighting how basic amenities directly impact technological advancements. Ultimately, the findings stress the necessity for comprehensive policies, training programs, and seamless system integration to foster transparency, accountability, and citizen trust—cornerstones for a more participatory and well-functioning Governance framework.

## FINDINGS

1. Low levels of digital literacy and awareness among citizens and government officials pose a challenge to effective e-Governance utilization.
2. Remote or marginalized communities face difficulties accessing e-Governance services, creating disparities in service availability.
3. Protecting citizen data and ensuring secure transactions within e-Governance systems is crucial, especially in sensitive regions like Jammu and Kashmir.
4. Extended power outages averaging 30 days per year significantly hinder the sustainability of e-Governance initiatives, leading to service disruptions and hampered accessibility, particularly in areas like Anantnag, where consistent power supply is crucial for the seamless functioning of digital systems.
5. Successful implementation requires strong policy support, governmental commitment, and adequate budget allocations, impacting the effectiveness of e-Governance initiatives in Anantnag.
6. Addressing diverse cultural and linguistic backgrounds within Anantnag presents a challenge in designing inclusive e-Governance platforms accessible to all.
7. Integration and compatibility among different e-Governance systems and platforms used by various government departments can be a significant hurdle.

8. Building trust among citizens regarding the reliability and efficacy of e-Governance systems is crucial for their widespread acceptance and use.
9. Training programs and skill development initiatives are essential to empower government officials and citizens to effectively utilize e-Governance tools and services.
10. Ensuring the sustainability and scalability of e-Governance projects over time and their expansion to cover more services and areas within Anantnag is a key challenge.
11. e-Governance promotes transparency by providing easy access to information, fostering accountability, and enhancing citizen-government trust, crucial for good Governance .
12. The interactions facilitate smoother communication between government, businesses, citizens, and employees, fostering transparency by enabling accessible information flow across administrative processes.
13. Frequent internet blackouts, totaling 422 between 2012 and 2022, pose a significant challenge, disrupting e-Governance services and communication channels.
14. Armed conflict and challenging geographical terrains further impede the implementation and accessibility of e-Governance initiatives, particularly in conflict-prone regions like Jammu and Kashmir
15. Ongoing efforts signal progress in comprehensive e-Governance , demanding sustained endeavors for broader accessibility and continual improvements.

**Suggestions for improvement**

1. Launch comprehensive digital literacy programs targeting citizens and government officials to empower them with necessary e-Governance skills.
2. Develop specialized outreach strategies for remote or marginalized communities, ensuring equitable access to e-Governance services.
3. Implement robust security protocols to safeguard citizen data, particularly in sensitive regions like Jammu and Kashmir.
4. Introduce alternative power solutions to mitigate the impact of frequent power outages on e-Governance initiatives in areas like Anantnag.
5. Advocate for strong policy support, governmental commitment, and adequate budget allocation to fortify e-Governance effectiveness.
6. Design e-Governance platforms considering diverse cultural and linguistic backgrounds within Anantnag for inclusivity.
7. Resolve compatibility issues among government e-Governance systems for streamlined operations.
8. Invest in campaigns to build citizen trust in e-Governance systems for widespread adoption.
9. Establish comprehensive training programs to empower officials and citizens in utilizing e-Governance tools effectively.
10. Develop long-term plans for the scalability and sustainability of e-Governance projects in Anantnag.
11. Emphasize e-Governance 's role in promoting transparency, accountability, and citizen-government trust.
12. Foster improved communication channels between government entities, businesses, citizens, and employees for transparent information flow.
13. Devise strategies to mitigate the impact of frequent internet blackouts on e-Governance services.
14. Tailor e-Governance strategies to navigate challenges in conflict-prone regions like Jammu and Kashmir.

## CONCLUSION

Electronic Governance , a technological blessing, fosters transparent, efficient Governance . Leveraging technology, it enables streamlined interactions between governments, businesses, citizens, and employees, ensuring accessibility, accountability, and improved service delivery for all stakeholders involved. The evolution from traditional Governance to e-Governance in Jammu and Kashmir represents a monumental technological shift facilitated by key agencies such as Jammu and Kashmir e -Governance agency, Common Service Centres (CSCs), Khidmat Centres, Software Technology Parks, and National Informatics Centre offices. These entities have played pivotal roles in aligning with the National e-Governance Plan (NeGP) to ensure the implementation of e-Governance practices uniformly across all districts. The transition has brought about significant changes, including amendments to legislation such as the Jammu and Kashmir Public Services Guarantee Act of 2011, specifically tailored to accommodate and enhance e-Governance service delivery mechanisms. The incorporation of various interactions, including Government-to-Business (G2B), Government-to-Citizen (G2C), Government-to-Government (G2G), and Government-to-Employee (G2E), has notably amplified transparency and accountability within administrative processes. Despite these strides, persistent challenges impede the full realization of e-Governance 's potential in the region. Notably, frequent internet blackouts, amounting to approximately 422 instances between 2012 and 2022, have severely disrupted digital connectivity. Rural areas suffer from limited access, contributing to the overarching digital divide. The presence of armed conflict and the complex, terrain-based geography pose formidable barriers to seamless e-Governance implementation. Addressing these challenges requires immediate and comprehensive solutions. Rectifying issues related to internet connectivity disruptions, especially the frequent blackouts, is imperative. Bridging the digital divide by ensuring equitable access to digital infrastructure in rural areas becomes paramount. The strategies must navigate the complexities posed by armed conflict and geographical constraints to ensure the uninterrupted provision of e-Governance services. Resolving these hurdles is crucial not only for the continued advancement of e-Governance but also for fostering inclusive development, equitable access to services, and transparent Governance practices throughout Jammu and Kashmir. Such solutions will play a pivotal role in creating an environment conducive to comprehensive and efficient e-Governance implementation across the region.

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**Table 1: e- Governance service**

| Service Provided | Description |
|---|---|
| Issuance of Birth Certificates | Providing official birth records within a specified time frame. |
| Issuance of Residence Certificates | Delivering residency proof documents within a defined period. |
| Application for Ration Cards | Processing applications and issuing ration cards within a specified timeframe. |

| | |
|---|---|
| Issuance of Driving Licenses | Providing driving permits within a stipulated time. |
| Utility Bill Payments | Ensuring timely processing of utility bill payments and acknowledgments. |
| Pension Disbursement | Ensuring pension payments are processed and disbursed on time. |
| Land Record Verification | Providing verified land records and documentation within a specified period. |
| Educational Scholarship Applications | Processing scholarship applications within defined timelines. |
| Building Permit Issuance | Granting building permits or approvals within a specified time frame. |

**Table 2 : SWOT Analysis**

| Strengths | Weaknesses | Opportunities | Threats |
|---|---|---|---|
| Increased Connectivity | Infrastructural Challenges | Rural Outreach Initiatives | Cyber security Vulnerabilities |
| Government Support | Digital Divide | Technological Advancements | Resistance to Technological Adoption |
| Citizen Engagement Tools | Limited Internet Penetration | Tourism & Economic Development | Political Instability |
| Skilled IT Workforce | Language Barriers | Investment in IT Infrastructure | Natural Disasters |

**Table 3: Electronic Governance tools for people and Government**

| Tools Used by People | Tools Used by Government |
|---|---|
| Online Portals/Websites | Online Portals/Websites |
| Mobile Applications | Mobile Applications |
| Digital Payment Systems | Digital Payment Gateways |
| National Identity Systems | Digital Identity Systems (e.g., Aadhaar) |
| Social Media Platforms | Data Analytics for Policy Planning |
| Self-Service Kiosks | Geographic Information Systems (GIS) |
| Customer Service Hotlines | Cyber security Measures |
| E-signatures | Cloud Computing for Data Storage |
| Online Chat Support | Block chain Technology (in some cases) |
| Public Wi-Fi | Artificial Intelligence (AI) and Chat bots |

**Table 4: Type of Interaction**

| Type of Interaction | Description | Example |
|---|---|---|
| Government-to-Business (G2B) | Processes involving government and business entities, such as licensing, taxation, and procurement. | Online application for a business license or bidding for a government contract. |
| Government-to-Citizen (G2C) | Interactions between government and individual citizens for services, information dissemination, and feedback. | Applying for a passport, accessing information on government schemes. |
| Government-to-Government (G2G) | Communication and data exchange between different government departments or agencies. | Sharing data between health and education departments for policy formulation. |
| Government-to-Employee (G2E) | Interactions catering to internal government employee processes, training, and communication within the workforce. | Employee payroll management, accessing training modules. |

**Year wise report of Internet Shut downs in Kashmir**

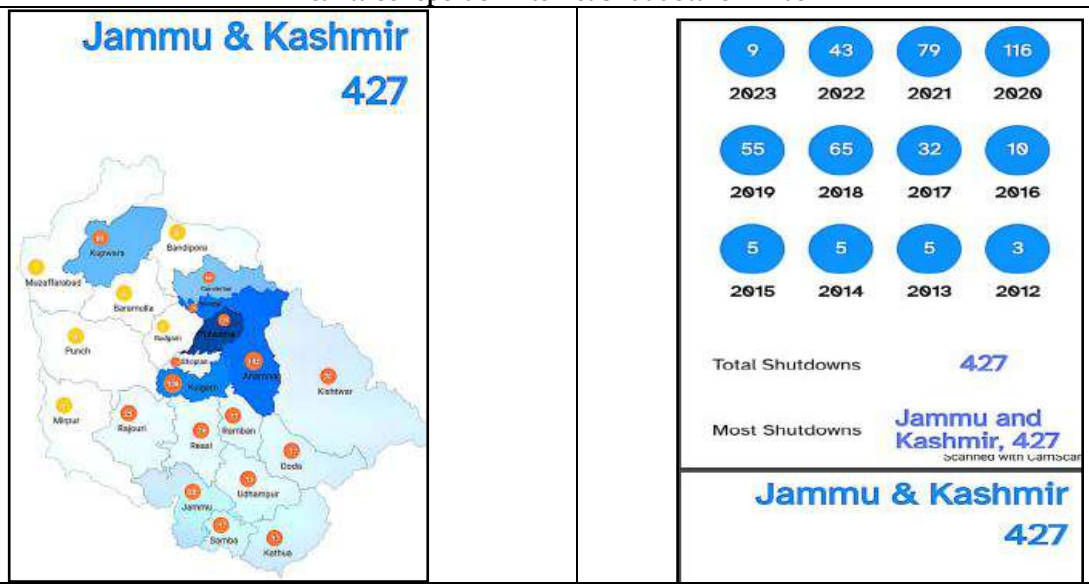


**Figure.1 : Year wise report of Internet Shut downs in Kashmir**
**Source: Government of Jammu and Kashmir Home department order no home- 41 TSTS of 2022 retrieved from https://internetshutdowns.in/Governmentstatic-page/jammu-kashmir**

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**RESEARCH ARTICLE**

# Human-Robot Collaboration - Optimizing Interaction in Industrial and Service Robots

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## ABSTRACT

Human-robot collaboration (HRC) is a burgeoning field that holds immense potential for revolutionizing both industrial and service sectors. This paper provides an overview of the current state and future directions in optimizing interaction between humans and robots in various domains. In industrial settings, collaborative robots, or "cobots" are increasingly deployed alongside human workers to enhance productivity and efficiency. These robots are equipped with advanced sensors, control algorithms, and safety features to ensure safe and effective interaction. Cobots assist in tasks such as assembly, material handling, and quality inspection, allowing human workers to focus on more complex and creative aspects of production. The success of human-robot collaboration hinges on seamless and intuitive interaction between humans and robots. This is facilitated by advancements in natural language processing, graphical user interfaces, and augmented reality, among other technologies. Despite significant progress, challenges remain in optimizing HRC, including ensuring safety and reliability, understanding human factors and ergonomics, addressing ethical and social implications, and developing scalable and flexible systems.

**Keywords:** Human-robot collaboration, Industrial robots, Service robots, Cobots, Interaction optimization, Collaborative robotics, Human-machine interaction.

## INTRODUCTION

Human-robot collaboration (HRC) is an emerging and transformative field within robotics and human-machine interaction. As the boundaries between human tasks and robotic automation blur, the need for efficient, safe, and intuitive collaboration mechanisms becomes paramount. This collaborative paradigm extends beyond mere co-existence, emphasizing synergistic interactions where both humans and robots bring their unique strengths to the table, resulting in enhanced productivity, safety, and flexibility across various domains. In industrial settings, robots have traditionally been confined to performing repetitive, hazardous, or precision-intensive tasks in isolation from human workers. The advent of collaborative robots, or "cobots," has revolutionized this landscape by enabling robots to work alongside humans on the factory floor. Cobots are designed with advanced sensors, control algorithms, and safety features to ensure safe and effective interaction. They assist in assembly, material handling, quality inspection, and other tasks, relieving human workers from physically demanding or monotonous duties and allowing them to focus on more complex and creative aspects of production. Service robots operate in environments where direct interaction with humans is essential. These robots are increasingly utilized in healthcare, domestic settings, customer service, and public spaces. For instance, in healthcare, robots assist with patient rehabilitation, elderly care, and surgical procedures. In domestic environments, robots perform household chores, enhancing the quality of life for their users. Customer service robots, on the other hand, engage with customers in retail and hospitality sectors, providing information, entertainment, and assistance. The optimization of human-robot interaction in these contexts is crucial for ensuring user acceptance, safety, and satisfaction.

## LITERATURE REVIEW

This review provides an overview of the applications, technologies, and challenges associated with service robots in industrial settings. It examines recent advancements in robot design, sensing, navigation, and human-robot interaction, highlighting their impact on improving efficiency, safety, and productivity in manufacturing and logistics operations [5]. This survey explores the state-of-the-art in human-robot collaboration (HRC) for industrial automation. It reviews research contributions in collaborative robotics, safety mechanisms, task allocation strategies, and ergonomic design considerations. The paper also discusses challenges and future directions for HRC implementation in manufacturing environments [6]. This article reviews recent developments in autonomous mobile robots (AMRs) for industrial applications. It examines AMR technologies such as navigation, mapping, localization, and fleet management systems. The paper discusses case studies and practical examples of AMR deployment in warehousing, logistics, and manufacturing facilities [7].

This review paper surveys the state-of-the-art in robotic systems for collaborative manufacturing. It discusses different types of collaborative robots (cobots), including their design principles, control strategies, safety features, and applications in assembly, machining, and material handling processes. The paper also highlights challenges and opportunities for future research in this area [8]. This paper examines the integration of service robots into Industry 4.0 environments. It discusses the role of robots in smart factories, digital manufacturing, and cyber-physical systems. The paper explores key challenges such as interoperability, security, and data privacy, and proposes solutions to enable seamless integration of service robots with other industrial technologies [9]. This study investigates safety considerations for human-robot collaboration in industrial environments. It reviews safety standards, risk assessment methods, and safety mechanisms implemented in collaborative robotic systems. The paper discusses factors influencing human-robot interaction safety and provides guidelines for ensuring safe and effective collaboration in manufacturing settings [10].

This systematic literature review examines the impact of service robots on manufacturing performance. It analyzes empirical studies and case reports to evaluate the effects of robot adoption on productivity, quality, cost, and flexibility in industrial operations. The paper identifies factors influencing the success of service robot implementation and provides insights for future research and practice [11]. This review paper discusses emerging

trends in service robotics for industrial automation. It covers topics such as collaborative robots, autonomous mobile robots, aerial drones, and wearable exoskeletons. The paper examines advancements in robot design, sensing technologies, and human-robot interaction, and explores their implications for improving efficiency and flexibility in manufacturing processes [12]. This review provides an overview of the challenges and opportunities of service robots in manufacturing. It discusses technical challenges such as robot perception, motion planning, and task allocation, as well as non-technical challenges related to cost, scalability, and regulatory compliance. The paper identifies research gaps and suggests future directions for advancing service robot technologies in manufacturing [13]. This comprehensive review examines the role of service robotics in various industrial sectors. It covers topics such as warehouse automation, logistics, healthcare, agriculture, and construction. The paper discusses the design, implementation, and performance evaluation of service robots in different application domains, highlighting their impact on improving efficiency, safety, and sustainability in industrial operations [14].

**Working Principle of Service Robot In The Industries**

Service robots are designed to perform tasks and provide assistance in various service-oriented environments, such as healthcare facilities, hospitality venues, retail stores, and domestic settings. The working methods of a service robot depend on its specific application and functionalities, but generally involve the following components and methods,

**Sensing and Perception**

**Environment Perception:** Service robots use sensors such as cameras, LiDAR, ultrasonic sensors, and depth sensors to perceive their surroundings. This allows them to navigate autonomously, avoid obstacles, and identify objects or people in their vicinity.

**Object Recognition:** Service robots employ computer vision techniques to recognize and classify objects, enabling them to interact with their environment and perform tasks such as fetching items, delivering packages, or assisting with inventory management.

**Navigation and Localization**

**Mapping:** Service robots create maps of their operating environment using simultaneous localization and mapping (SLAM) algorithms. This helps them navigate efficiently and avoid collisions by planning optimal paths to reach their destinations.

**Localization:** Robots use localization techniques, such as GPS, WiFi triangulation, or visual landmarks, to determine their position within the environment and update their maps accordingly.

**Human-Robot Interaction (HRI)**

**Speech Recognition:** Service robots integrate natural language processing (NLP) algorithms to understand spoken commands and respond appropriately to user queries or requests.

**Gesture Recognition:** Some service robots can interpret human gestures, such as pointing or waving, as input commands, allowing for intuitive interaction with users.

**Touch screen Interfaces:** Robots equipped with touchscreen displays or graphical user interfaces (GUIs) enable users to interact with them using touch-based input methods, such as tapping or swiping.

**Task Execution and Manipulation**

**Manipulation:** Service robots equipped with robotic arms or grippers can manipulate objects to perform tasks such as picking up items, placing them in designated locations, or assisting with assembly tasks.

**Mobility:** Mobile service robots can move autonomously or be teleoperated to navigate within indoor or outdoor environments, allowing them to reach different locations and perform diverse tasks as needed.

**Payload Capacity:** The payload capacity of a service robot determines its ability to carry and transport objects or equipment, making it suitable for tasks such as delivering goods, serving meals, or transporting medical supplies.

**Data Processing and Decision Making**

**Onboard Computing:** Service robots are equipped with onboard computers or processors to process sensor data, execute control algorithms, and make decisions in real-time.

**Machine Learning:** Some service robots incorporate machine learning techniques to adapt and improve their performance over time based on experience and feedback from interactions with users and the environment.

**Safety and Emergency Features**

**Collision Avoidance:** Service robots employ collision detection and avoidance mechanisms to navigate safely in crowded or dynamic environments, preventing accidents or injuries.

**Emergency Stop:** Robots are equipped with emergency stop buttons or sensors that allow users or operators to halt their operation in case of emergencies or unexpected situations.

**Connectivity and Integration**

**Network Connectivity:** Service robots may be connected to the internet or local networks to access cloud services, receive software updates, or communicate with other devices and systems.

**Integration with IoT Devices:** Robots can integrate with Internet of Things (IoT) devices and smart home appliances to perform coordinated tasks or provide enhanced services, such as controlling lights, thermostats, or security cameras.

**Maintenance and Self-diagnosis**

**Diagnostic Sensors:** Robots incorporate diagnostic sensors and self-check mechanisms to monitor their health status, detect faults or malfunctions, and alert users or maintenance personnel when repairs or servicing are required.

**Remote Monitoring:** Some robots support remote monitoring and management capabilities, allowing operators to track their status, performance, and usage patterns from a centralized dashboard or management interface.

By employing these working methods and capabilities, service robots can effectively perform a wide range of tasks and provide valuable assistance in diverse service-oriented environments, enhancing efficiency, productivity, and user satisfaction.

**Drawbacks of Service Robots In The Industries**

Service robots offer promising solutions for enhancing productivity and efficiency in industrial environments, yet they come with several inherent limitations. These include challenges related to the complexity of tasks, adaptability to changing requirements, cost considerations, and safety concerns regarding human-robot interaction. Additionally, achieving true autonomy, seamless integration with existing systems, and ensuring maintenance reliability remain ongoing hurdles. Ethical and social implications, such as job displacement and environmental impact, also warrant careful consideration. Overcoming these limitations requires concerted efforts in research and development, collaboration among stakeholders, and the implementation of robust regulatory frameworks to ensure the safe and responsible deployment of service robots while maximizing their benefits in industrial settings.

## CONCLUSION

Despite significant progress, challenges persist in optimizing HRC. Ensuring safety and reliability, understanding human factors and ergonomics, addressing ethical and social implications, and developing scalable and flexible systems remain key areas of focus for researchers and practitioners. In conclusion, human-robot collaboration holds immense promise for transforming industrial and service sectors by leveraging the complementary strengths of humans and robots. By harnessing technological advancements and addressing critical challenges, HRC has the potential to enhance productivity, safety, and user satisfaction, paving the way for a future where humans and robots work together synergistically to achieve common goals. Through continued research, innovation, and collaboration, we can unlock the full potential of human-robot collaboration, ushering in a new era of intelligent automation and human-machine interaction.

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**RESEARCH ARTICLE**

# Impact of Organic Nutrient Management on Nutrients Uptake and Soil Nutrients Availability at Different Growth Stages of Kodo Millet

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## ABSTRACT

The present study was carried out at Farmer's Field, Vallampadugai Village, Chidambaram Taluk in Cuddalore District during August – November, 2022 to investigate soil application of bio-compost, consortia bio-fertilizer and foliar spray of panchagavya and fish amino acid on nutrients uptake by kodo millet and nutrients availability in sandy loam soil. The field trial was laid out in Randomized Block Design (RBD) with nine treatments and three replications. The study included nine treatments were $T_1$- Absolute control, $T_2$- 100% RDF (44:22:0 kg N: $P_2O_5$: $K_2O$ $ha^{-1}$), $T_3$-Bio-compost @ 10 t $ha^{-1}$, $T_4$- $T_3$+ CBF@ 2 kg $ha^{-1}$, $T_5$ -$T_3$+ PK@3%, $T_6$-$T_3$+ FAA@0.5%, $T_7$-$T_3$+ CBF@ 2 kg $ha^{-1}$ +PK@3%, $T_8$-$T_3$+ CBF@ 2 kg $ha^{-1}$ + FAA @ 0.5%, $T_9$-$T_3$+PK@3%+FAA @ 0.5%. Kodo millet was grown as test crop with proper cultural practices. The results of the study revealed that nutrients uptake *viz.*, N, P and K by kodo millet significantly increased from 35 DAS to harvest stages due to different treatments. At harvest stage, the highest N uptake (34.86 kg $ha^{-1}$), P uptake (9.79 kg $ha^{-1}$) and K uptake (36.30 kg $ha^{-1}$) were found to be with $T_7$. However, lowest N, P and K uptake values were significantly lowest with control ($T_1$). Application of bio-compost @10 t $ha^{-1}$ + CBF@ 2 kg $ha^{-1}$+PK@3% ($T_7$) significant effect on soil available N, P and K status at all the stages compared to control. The highest post-harvest soil available N(194 $kgha^{-1}$), P(11.6 $kgha^{-1}$) and K(226 $kgha^{-1}$) status were noticed in $T_7$ - Bio-compost @10 t $ha^{-1}$ + CBF@ 2 kg $ha^{-1}$+PK@3% than control ($T_1$).

**Keywords:** Nutrients uptake, soil nutrients availability, kodo millet and organic nutrients

## INTRODUCTION

Kodo millet (*Paspalum scrobiculatum L.*) is a tropical small millet originated in tropical Africa and it has been cultivated in India 3000 years ago. Its grain contains 66.6% carbohydrate, 8.35% protein, 2.4% minerals, 1.45% fat and 2.95% ash. The continuous intake of kodo millet prevents cardiovascular diseases and reducing the blood pressure and high cholesterol (Bunkar *et al.,* 2021). Organic farming is a production system that sustains soil health, ecosystem and people by relaying on ecological processes, biodiversity and natural cycles and adapted to local conditions than use of inputs with adverse effects. It lies in a simple principle of utilizing cheap and local inputs with zero utilization of chemicals (Raghuvaran singh *et al.,* 2021). Bio-compost is a fluffy dark brown to black organic by product of sugarcane juice processing and contains organic nutrients, sugars, wax and other bio-chemical constituents. Consortium bio-fertilizers are eco-friendly, cost effective and renewable sources of plant nutrients that help to maintain long term soil fertility and sustainability. Panchagavya possesses major nutrients *viz.,* N,P,K and micro nutrients and growth hormones *viz.,* IAA and GA required for crop growth (Natarajan , 2007). It is also known to boost immunity and promote plant growth. Fish amino acid is of great value to both plants and microbes because it contains various nutrients and types of amino acids that is essential for the growth and yield of crops. The study aimed to find out the effect of bio-compost, consortium bio-fertilizer, panchagavya and fish amino acid on nutrients uptake by kodo millet and nutrients availability in sandy loam soil.

## MATERIALS AND METHODS

The field experiment was carried out at Farmer's Field, Vallampadugai Village, Chidambaram Taluk in Cuddalore District during August– November, 2022 to investigate the effect of soil application of bio-compost, consortia bio-fertilizer and foliar application of panchagavya and fish amino acid on nutrients uptake by kodo millet and nutrients availability in *Typic Ustifluvent* soil. The soil of the experimental field was sandy loam in texture, Vertisols in order with the taxonomic classification of *Typic Ustifluvent*. The pH, EC of the experimental soil were 7.38 and 0.32, respectively. The field experiment consists of nine treatments and three replications in randomized block design (RBD). The treatments were $T_1$ - Absolute Control: $T_2$ - 100% RDF: $T_3$ - Bio-compost @ 10 t ha$^{-1}$: $T_4$ - $T_3$+ CBF@ 2 kg ha$^{-1}$: $T_5$ - $T_3$+ PK@3% : $T_6$ - $T_3$+ FAA@0.5% : $T_7$ - $T_3$+ CBF@ 2 kg ha$^{-1}$ + PK@3% : $T_8$ - $T_3$+ CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5% and $T_9$ - $T_3$+PK@3%+FAA @ 0.5%. These were imposed randomly on the plots in each replication. The recommended dose of fertilizers (44:22:0 kg N: $P_2O_5$: $K_2O$ ha$^{-1}$) were applied to the field through Urea, SSP and MOP, respectively. The full dose of nitrogen and phosphorus were given as basal. Based on treatments, bio-compost @ 10 t ha$^{-1}$, consortia bio-fertilizer@ 2 kg ha$^{-1}$ were applied one week before sowing. As per the treatment schedule, panchagavya@3% and fish amino acid @ 0.5% sprayed twice (30 DASand 60 DAS) during vegetative and reproductive stages of kodomillet. The kodo millet cv. CO-3 seeds were treated with consortia bio- fertilizer @ 600 g ha$^{-1}$ and sown with the spacing of 45x10 cm. The plant protection measures were taken with neem seed kernel extract. The test crop was grown with standard cultural practices. The effect of treatments on nutrients uptake and soil nutrients availability at different growth stages of kodomillet were analyzed with standard procedures and recorded.

## RESULTS AND DISCUSSION

### NUTRIENTS UPTAKE

**Nitrogen uptake (kg ha$^{-1}$)**

The data pertaining to nitrogen, phosphorus and potassium uptake by kodo millet as influenced by soil application of bio compost, consortia bio fertilizer and foliar spray of panchagavya and fish amino acid was significant and given in table 1. At 35 DAS, application of bio compost@10 t ha$^{-1}$ +CBF@2 kg ha$^{-1}$ +PK@3% ($T_7$) recorded highest nitrogen uptake of 13.39 kg ha$^{-1}$. This was followed by $T_8$ -Bio compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5 % (12.18 kg ha$^{-1}$) and $T_9$ (bio compost @10 t ha$^{-1}$+PK@3% +FAA @ 0.5%) (11.85 kg ha$^{-1}$) which were on par. The treatments

$T_2$, $T_5$ and $T_6$ registered nitrogen uptake of 10.61, 9.42 and 9.35 kg ha$^{-1}$, respectively. However lowest nitrogen uptake of 4.81 kg ha$^{-1}$ was found to be with control ($T_1$).

At 70 DAS, significantly highest nitrogen uptake by kodo millet was 35.70 kg ha$^{-1}$ found to be with bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + PK@3% ($T_7$). It was followed by 32.02 and 31.35 kg ha$^{-1}$ were found to be with $T_8$ (Bio - compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5 % ) and $T_9$ (Bio - compost @10 t ha$^{-1}$+PK@3% +FAA @ 0.5%). Application of 100% RDF (44:22:0 kg N: $P_2O_5$: $K_2O$ ha$^{-1}$) registered nitrogen uptake of 27.71 kg ha$^{-1}$ respectively. However, control ($T_1$) recorded lowest nitrogen uptake (10.25 kg ha$^{-1}$).

At harvest stage, highest nitrogen uptake of 17.11 kg ha$^{-1}$ by grain was recorded with $T_7$ which received bio - compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + PK@3%.This was followed by $T_8$,$T_9$, $T_2$, $T_5$ and $T_6$ registered grain nitrogen uptake of 15.68,15.19, 13.80, 12.33 and 12.14 kg ha$^{-1}$, respectively. The treatments $T_8$and $T_9$and $T_5$ and $T_6$ were on par. However, least nitrogen uptake (7.05 kg ha$^{-1}$) was observed under control ($T_1$) which was received no organic and inorganic nutrients sources. At harvest stage, nitrogen uptake (34.86 kg ha$^{-1}$) by straw was found to be superior with application of bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$). It was followed by 31.72 and 31.19 kg ha$^{-1}$ were found to be with $T_8$ (Bio compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5 %) and $T_9$ (Bio compost @10 t ha$^{-1}$+PK@3% +FAA @ 0.5%). Application of 100% RDF (44:22:0 kg N: $P_2O_5$: $K_2O$ ha$^{-1}$) registered nitrogen uptake by straw was 28.08 kg ha$^{-1}$. However, control ($T_1$) recorded lowest nitrogen uptake of 12.24 kg ha$^{-1}$. This might be due to the application of bio-compost which had higher content of nitrogen to be mineralized and made available for the crop uptake. This is confirmed with the findings of Sharma *et al.* (2017).The increased nutrient uptake might be also due to more accumulation of dry matter with increased plant population and crop geometry. The presence of chemolithotrophs and autotrophic nitrifiers in panchagavya, which colonize the leaves, enhanced ammonia uptake and increased the total nitrogen uptake by kodo millet (Papen, 2002).

**Phosphorous uptake (kg ha$^{-1}$)**

Similar to nitrogen uptake, phosphorus uptake by kodo millet also showed significant differences among treatments. At 35 DAS, application of bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$) recorded highest phosphorous uptake of 4.24 kg ha$^{-1}$. This was followed by $T_8$- Bio compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5 % (3.87 kg ha$^{-1}$) and $T_9$ (Bio compost @10 t ha$^{-1}$+PK@3% +FAA @ 0.5%) (3.73 kg ha$^{-1}$) which were on par. The treatments $T_2$, $T_5$ and $T_6$ registered phosphorous uptake of 3.35, 2.95 and 2.86 kg ha$^{-1}$, respectively. However lowest phosphorous uptake of 1.53 kg ha$^{-1}$ was found to be with control ($T_1$). At 70 DAS significantly highest phosphorous uptake by kodo millet 10.64 kg ha$^{-1}$ was found to be with bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$). It was followed by 9.58 and 9.52 kg ha$^{-1}$ were found to be with $T_8$ and $T_9$. However, control ($T_1$) recorded lowest phosphorous uptake (3.24 kg ha$^{-1}$). At harvest stage, highest phosphorous uptake by grain was 4.60 kg ha$^{-1}$found to be with $T_7$. This was followed by $T_8$,$T_9$, $T_2$, $T_5$ and $T_6$ registered phosphorous uptake by grain were 4.21,4.17, 3.75, 3.34 and 3.27 kg ha$^{-1}$, respectively. The treatments $T_8$ and $T_9$; $T_5$ and $T_6$ were on par. However, least phosphorous uptake by grain (1.71 kg ha$^{-1}$) was observed under control ($T_1$). At harvest stage, phosphorous uptake by straw (9.79 kg ha$^{-1}$) was found to be superior with application of bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + PK@3% ($T_7$). It was followed by 8.96 and 8.79 kg ha$^{-1}$ were found to be with $T_8$ and $T_9$. However, control ($T_1$) recorded lowest phosphorous uptake (4.23 kg ha$^{-1}$).

This increased phosphorus uptake might be due to increased dry matter, yield as well as P content. The higher uptake of nutrients might have attributed to greater DMP and better root growth (Dhananivetha, 2016). This increase might be also due to the favourable influence in reducing the fixation of P and subsequently enhancing the dry matter production. The orthophosphate ion might have converted from $PO_4^{3-}$ to $HPO_4^{2-}$ for short periods which resulted in increased concentration of P in plant. These results are in accordance with the observation of Tilahun *et al.* (2013).

**Potassium uptake (kg ha$^{-1}$)**

Among the various treatments tried, at 35 DAS, application of bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$) recorded highest potassium uptake of 14.57 kg ha$^{-1}$. This was followed by $T_8$(13.21 kg ha$^{-1}$) and $T_9$ (12.75 kg ha$^{-1}$)were on par. The treatments $T_2$, $T_5$ and $T_6$ registered potassium uptake of 5.36, 11.27 and 10.88 kg ha$^{-1}$respectively. However lowest potassium uptake of 4.98 kg ha$^{-1}$ was found to be with control ($T_1$). At 70 DAS, significantly highest potassium uptake by kodo millet 37.14 kg ha$^{-1}$ was found to be with $T_7$ and control ($T_1$) recorded lowest potassium uptake of 11.45 kg ha$^{-1}$.

At harvest stage, highest potassium uptake by grain was 17.94 kg ha$^{-1}$ recorded in the treatment $T_7$ which was received bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3%.This was followed by $T_8$ ,$T_9$, $T_2$, $T_5$ and $T_6$ registered potassium uptake by grain were 16.59,16.31, 8.83, 14.87 and 14.59 kg ha$^{-1}$, respectively. However, least potassium uptake by gain (8.04 kg ha$^{-1}$) was observed under control ($T_1$). At harvest stage, potassium uptake by straw (36.30 kg ha$^{-1}$) was found to be superior with application of bio compost@10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$). However, control ($T_1$) recorded lowest potassium uptake (16.20 kg ha$^{-1}$). The higher K uptake might be due to faster rate of mineralization and greater utilization of nutrients through use of bio-compost. The higher potassium uptake with bio-compost might be attributed to solubilisation of native nutrients, chelation complex of intermediate organic molecules produced during decomposition of added manures with nutrients their mobilization and accumulation of nutrients in different plant parts (Chesti *et al.,* 2015)

**SOIL NUTRIENTS AVAILABILITY**

**Soil available nitrogen (kg ha$^{-1}$)**

The data on soil available nitrogen status recorded at various growth stages (35, 70 DAS and at harvest stage) as influenced by different combinations of organic nutrient sources *viz.*, bio - compost, consortia bio - fertilizer, panchagavya and fish amino acid are presented in table 2. At 35 DAS, significantly highest soil available nitrogen status of 242 kg ha$^{-1}$ was recorded with application of bio-compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + PK@3% ($T_7$). The second highest soil available nitrogen status of 238 kg ha$^{-1}$ was observed with $T_8$. It was on par with application of bio-compost +PK@3%+FAA @ 0.5% ($T_9$) registered soil available nitrogen status of 235 kg ha$^{-1}$. However, lowest value of 210 kg ha$^{-1}$ was observed under control ($T_1$)

At 70 DAS, soil available nitrogen status ranged from 185 to 220 kg ha$^{-1}$. Application of bio-compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$) significantly increased soil available nitrogen status of 220 kg ha$^{-1}$ over control recorded lowest soil available nitrogen status of 185 kg ha$^{-1}$. At harvest stage, available nitrogen status range between 164 to 194 kg ha$^{-1}$. The highest soil available nitrogen status (194 kg ha$^{-1}$) was noticed in $T_7$ whereas lowest (164 kg ha$^{-1}$)was observed in $T_1$ (control). Application of bio-compost + CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5% ($T_8$) and bio compost +PK@3%+FAA @ 0.5% ($T_9$) registered soil available nitrogen status of 190 and 186 kg ha$^{-1}$, respectively. The higher microbial activity facilitates the conversion of organically bound nitrogen to inorganic nitrogen, consistent with the findings of Mairan and Dhawan (2018) and Yogananda(2019). The highest soil nitrogen availability could be attributed to the favourable environment for soil organisms involved in nitrogen transformation with bio-compost (Yang *et al.,* 2013).

**Soil available phosphorus (kg ha$^{-1}$)**

At 35 DAS, significantly highest soil available phosphorus status of 14.9 kg ha$^{-1}$ was recorded with $T_7$. The second highest phosphorus status of 14.0 kg ha$^{-1}$ was observed with $T_8$. It was on par with $T_9$ registered phosphorus status of 13.8 kg ha$^{-1}$. However, lowest soil available phosphorus status of 9.2 kg ha$^{-1}$ was observed under control ($T_1$) At 70 DAS, soil available phosphorus status ranged from 7.7 to 12.7 kg ha$^{-1}$. Application of bio-compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$+PK@3% ($T_7$) significantly increased soil available nitrogen status of 12.7 kg ha$^{-1}$ over control (12.7 kg ha$^{-1}$).

At harvest, lowest soil available phosphorus status of 7.1 kg ha$^{-1}$ was found to be with control ($T_1$). Whereas, highest soil available phosphorus status of 11.6 kg ha$^{-1}$ was observed in $T_7$. The treatments $T_8$ and $T_9$ registered soil available

phosphorus status of 11.2 and 11.0 kg ha$^{-1}$, respectively. Addition of bio-compost enhanced the available phosphorus results in mineralization of organic phosphates and the generation of organic acids through microbial decomposition of bio-compost contribute to the solubilization of native soil phosphates and organic amines, thereby inhibiting phosphorus (P) fixation in the soil. Similar results were observed by Roy (2018).

**Soil available potassium (kg ha$^{-1}$)**

At 35 DAS, significantly maximum soil available potassium status of 269 kg ha$^{-1}$ was recorded with application of bio-compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ + PK@3% ($T_7$). The second highest soil available potassium status of 261 kg ha$^{-1}$ was observed with $T_8$- Bio-compost + CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5%). It was on par with application of bio-compost +PK@3%+FAA @ 0.5% ($T_9$) (256 kg ha$^{-1}$). At 70 DAS, soil available potassium status ranged from 225 to 252 kg ha$^{-1}$. Application of bio-compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$) significantly increased soil available potassium status of 252 kg ha$^{-1}$compared to control (225 kg ha$^{-1}$).

At harvest, bio-compost @10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% ($T_7$) showed highest soil available potassium status (226 kg ha$^{-1}$).The treatments $T_8$, $T_9$ and $T_4$ registered soil available potassium status of 221, 220 and 219 kg ha$^{-1}$, respectively were statistically on par with each other. However, lowest (202kg ha$^{-1}$) was found to be with $T_1$. The incorporation of bio-compost reduced potassium fixing, leading to the eventual release of potassium. Additionally, the crops potassium demand was partially met by the potassium compounds released during decomposition. The applied and released potassium contributed to an increase in accessible potassium in the soil. These findings supports with studies conducted by Krishnamurthy *et al.* (2020).

## CONCLUSION

Among the different organic nutrient sources tried in the present investigation, the combination of soil application of bio-compost, consortia bio-fertilizer and foliar spray of panchagavya and fish amino acid ($T_7$) significantly increased NPK uptake by kodo millet all the growth stages compared to control. The same treatment ($T_7$) Bio-compost @ 10 t ha$^{-1}$ + CBF@ 2 kg ha$^{-1}$ +PK@3% significantly enhanced soil fertility status with respect to nitrogen, phosphorus and potassium availability in sandy loam soil.

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**Table 1: Effect of organic nutrient management on nitrogen, phosphorus and potassium uptake (kg ha$^{-1}$) by kodo millet *cv*. $CO_3$ at different growth stages**

| T. No. | Treatment Details | N uptake (kg ha $^{-1}$) | | | | P uptake (kg ha $^{-1}$) | | | | | | Harvest | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 35 DAS | 70 DAS | Harvest | | 35 DAS | 70 DAS | Harvest | | 35 DAS | 70 DAS | Harvest | |
| | | | | Grain | Straw | | | Grain | Straw | | | Grain | Straw |
| $T_1$ | Control | 4.81 | 10.25 | 7.05 | 12.24 | 1.53 | 3.24 | 1.71 | 4.23 | 4.98 | 11.45 | 8.04 | 16.20 |
| $T_2$ | 100% RDF | 10.61 | 27.71 | 13.80 | 28.08 | 3.35 | 8.43 | 3.75 | 7.98 | 5.36 | 12.58 | 8.83 | 16.64 |
| $T_3$ | Bio-compost @ 10 t ha$^{-1}$ | 6.59 | 15.37 | 9.03 | 16.98 | 2.05 | 5.11 | 2.43 | 5.52 | 7.66 | 16.99 | 11.86 | 20.29 |
| $T_4$ | $T_3$+ CBF@ 2 kg ha$^{-1}$ | 8.01 | 19.33 | 10.61 | 20.04 | 2.43 | 6.20 | 2.81 | 6.30 | 9.34 | 21.34 | 13.21 | 23.64 |
| $T_5$ | $T_3$+ PK@3% | 9.42 | 23.98 | 12.33 | 24.85 | 2.95 | 7.36 | 3.34 | 7.20 | 11.27 | 26.72 | 14.87 | 28.29 |
| $T_6$ | $T_3$+ FAA@0.5% | 9.35 | 23.19 | 12.14 | 23.71 | 2.86 | 7.28 | 3.27 | 7.09 | 10.88 | 25.62 | 14.59 | 27.12 |
| $T_7$ | $T_3$+ CBF@ 2 kg ha$^{-1}$ + PK@3% | 13.39 | 35.70 | 17.11 | 34.86 | 4.24 | 10.64 | 4.60 | 9.79 | 14.57 | 37.14 | 17.94 | 36.30 |
| $T_8$ | $T_3$+ CBF@ 2 kg ha$^{-1}$ + FAA @ 0.5% | 12.18 | 32.02 | 15.68 | 31.72 | 3.87 | 9.58 | 4.21 | 8.96 | 13.21 | 32.86 | 16.59 | 32.90 |
| $T_9$ | $T_3$ + PK@3% +FAA @ 0.5% | 11.85 | 31.35 | 15.19 | 31.19 | 3.73 | 9.52 | 4.17 | 8.79 | 12.75 | 30.93 | 16.31 | 31.67 |
| S.Ed | | 0.524 | 1.601 | 0.264 | 1.41 | 0.18 | 0.50 | 0.16 | 0.07 | 0.601 | 1.891 | 0.574 | 1.468 |
| CD (P = 0.05) | | 1.154 | 3.524 | 0.582 | 3.08 | 0.40 | 1.11 | 0.36 | 0.16 | 1.324 | 4.161 | 1.263 | 3.23 |

**Table 2: Effect of organic nutrient management on soil available nitrogen, phosphorus and potassium status (kgha$^{-1}$) at different growth stages of kodo millet *cv*. $CO_3$**

| T. No. | Treatment Details | Soil Available N (kg ha $^{-1}$) | | | Soil Available P (Kg ha$^{-1}$) | | | Soil Available K (Kg ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 35 DAS | 70 DAS | Harvest | 35 DAS | 70 DAS | Harvest | 35 DAS | 70DAS | Harvest |
| $T_1$ | Absolute Control | 210 | 185 | 164 | 9.2 | 7.7 | 7.1 | 237 | 225 | 202 |
| $T_2$ | 100% RDF | 222 | 196 | 170 | 11.7 | 9.7 | 8.6 | 239 | 228 | 205 |
| $T_3$ | Bio-compost @ 10 t ha$^{-1}$ | 228 | 208 | 178 | 13.1 | 11.3 | 10.1 | 250 | 235 | 211 |
| $T_4$ | $T_3$+ CBF@ 2 kg ha$^{-1}$ | 233 | 214 | 184 | 13.9 | 12.0 | 11.0 | 255 | 241 | 219 |
| $T_5$ | $T_3$+ PK@3% | 230 | 210 | 180 | 13.0 | 11.0 | 10.3 | 252 | 238 | 214 |
| $T_6$ | $T_3$+ FAA@0.5% | 229 | 209 | 179 | 12.9 | 11.1 | 10.2 | 251 | 236 | 213 |
| $T_7$ | $T_3$+ CBF@ 2 kg ha$^{-1}$ + PK@3% | 242 | 220 | 194 | 14.9 | 12.7 | 11.6 | 269 | 252 | 226 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $T_8$ | $T_3$+ CBF@ 2 kg $ha^{-1}$ + FAA @ 0.5% | 238 | 215 | 190 | 14.0 | 12.3 | 11.2 | 261 | 246 | 221 |
| $T_9$ | $T_3$ + PK @ 3% +FAA @ 0.5% | 235 | 211 | 186 | 13.8 | 12.1 | 11.0 | 256 | 242 | 220 |
| S.Ed | | 1.433 | 1.640 | 1.449 | 0.259 | 0.135 | 0.159 | 2.14 | 2.023 | 1.655 |
| CD (P = 0.05) | | 3.154 | 3.610 | 3.189 | 0.571 | 0.297 | 0.351 | 4.721 | 4.452 | 3.641 |

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**RESEARCH ARTICLE**

# Study on Genotoxic Potential of Commonly used Herbicide on *Clarias batrachus*

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## ABSTRACT

The genotoxic potential of the herbicide $CuSO_4$ was assessed using the micronucleus assay in *Clarias batrachus.* Two different methods were employed: intraperitoneal administration of three doses (1.5, 3.0,and 4.5 mg/kg body weight) and exposure to varying concentrations (15, 25, and 35 ppm) of copper sulfate in laboratory aquaria. Peripheral blood smears were stained with 15 to 20% Giemsa solution (pH=7.0). In addition to micronuclei, the chemical induced various nuclear and cytoplasmic abnormalities. The research revealed that elevated levels of $CuSO_4$ had a noticeable effect on the biological specimens of *Clarias batrachus.* This species is prevalent in various freshwater environments, including ponds, ditches, wetlands, and rice fields, especially within the region of Odisha, India. The widespread application of pesticides containing $CuSO_4$ in agricultural practices poses substantial hazards to *C. batrachus*, a species vital for preserving aquatic biodiversity. Distinctively, the investigation unveiled the adverse consequences of heightened $CuSO_4$ concentrations on *Clarias batrachus* specimens. This species, indigenous to fresh water ecosystems such as ponds, ditches, wetlands, and rice fields in Odisha, India, faces considerable jeopardy due to the indiscriminate utilization of $CuSO_4$-based pesticides in agriculture. This underscores the critical role of *C. batrachus* in upholding the ecological balance of aquatic habitats.

**Keywords:** *Clarias batrachus*, copper sulphate, micro nucleus assay, genotoxic potential, peripheral blood smear.

## INTRODUCTION

The previous three to four decades have seen a great expansion of the chemical industries due to various industrial development initiatives, as evidenced by the invention of numerous new chemical products and their growing application. These substances, which might be simple or complex and can be found in nature or synthetic materials, contaminate our food, water, and air when they are released into the environment. These substances may have negative effects on other living things that impact the ecosystem, which eventually puts human health and wellbeing in jeopardy. Ninety to ninety-five percent of all sewage and seventy percent of all industrial wastes are disposed of untreated into surface waters in underdeveloped nations (UNFPA, 2001). The potential of crop loss from agricultural pests grows as there is a limited supply of arable land to serve the world's growing population. Consequently, pesticides are now a necessary part of contemporary farming. Toxic chemicals known as pesticides are purposefully released into the environment to suppress unwanted organisms of all kinds. Many different pesticides are used to control pests, such as weeds, insects, mites, and nematodes, in the agricultural sector, industry, households, and public health and safety. The necessity for bio-monitoring terrestrial and aquatic ecosystems is urgent because of the rising environmental exposure to these agents, particularly in areas where chemical pollution is a problem. [Silva et al., 2003; Matsumoto et al., 2003 and 2005; Mitchelmore and Chipman, 1998; Avishai et al., 2002]. Genotoxicity is a harmful process that tampers with a cell's genetic material, compromising its integrity [Environ Health Perspective, 1996; WHO, 1997]. It is well known that a number of genotoxic substances are mutagenic and carcinogenic, particularly those that have the ability to cause genetic mutation and to have an impact on the growth of human tumors or cancers [Hayashi et al., 1998; Fagr et al., 2008; Shugart, 1988; Black et al., 1983; Hose, 1985; Baumann and Mac, 1988; Hose et al., 1984]. These include specific chemical substances such as microbiological toxins [Environ Health Perspect, 1996] and heavy metals [Matsumoto et al., 2005; Igwilo et al., 2006; Matsumoto,2003; Pruski and Dixon, 2002]. It has been demonstrated that a number of insecticides may be genotoxic to various freshwater fish. In *Clarias batrachus*, acephate results in nuclear abnormalities and damage to DNA [Jagyanseni et.al, 2022]. In *Heteropneustes fossilis*, acephate damages DNA and modifies hematological parameters [Jagyanseni et al., 2023]. Fish hematological characteristic evaluation has grown in importance as a tool for evaluating both normal and abnormal procedures and toxicological effects [Sudova, et al., 2009]. In *Channa punctatus*, a lower RBC and Hb concentration was generated by a sub lethal dose of $CuSO_4$ compared to the control group. Following treatment with varying concentrations of $CuSO_4$, it was noted that the amount of RBC in *C.macropomum* decreased [Gryphon et al., 1999]. The current study's objective was to assess, using a micronucleus assay, the genotoxic effects of copper sulphate on the freshwater fish *Clarias batrachus*.

## MATERIALS AND METHODS

**Test Chemical:** The study employed high-quality copper sulfate $CuSO_4$, $5H_2O$, with a purity of 99%, sourced from Fine-chem. (India) Ltd. Glass double-distilled (g.d.d) water served as the solvent in the experimentation process.

**Dose:** Intraperitoneal (i.p.) administration of three different doses (1.5, 3.0, and 4.5 mg/kg body weight) to *Clarias batrachus* was conducted. Additionally, another set of experiments involved exposure to 15, 25, and 35 ppm of copper sulfate in laboratory aquaria.

**Experimental animal:** Live *Clarias batrachus* weighing between 30 to 50 grams were obtained from local suppliers in Cuttack district. Before initiating any chemical treatments, these fish underwent a period of acclimatization to laboratory conditions. During this phase, they were housed in glass aquaria containing 30 liters of dechlorinated tap water for 5 to 6 days. The fish were provided with commercial fish food twice daily, and the aquarium water underwent aeration and daily replacement to uphold the desired physio-chemical parameters. These parameters included a temperature range of 28°C, a pH level between 6.8 and 7.05, and dissolved oxygen levels between 6.5 and 7.3 mg/L. Regular maintenance of the aquaria ensured the removal of left over feed, waste, and any deceased organisms to minimize stress and prevent contamination.

**TIME:** Blood smear slides for analysis were prepared at intervals of 24, 48, and 72 hours following exposure to the chemicals.

**Experimental Protocol:** Four fish individuals were allocated to each treatment group for both intraperitoneal and dermal administrations. Peripheral blood smear slides were prepared from blood collected via caudal incision at 24, 48, and 72- hour intervals. Thin smears of peripheral blood were made on clean, grease- free slides, fixed in absolute methanol, stained with Giemsa solution, and examined under oil immersion. Micronuclei, non-refractile particles resembling nuclei but of smaller size, were scored from 4000 erythrocytes per specimen. Micronuclei in *Clarias batrachus* varied in size from 1/5 to 1/28th of the main nucleus.

## RESULTS AND DISCUSSION

**General Toxicity:** After administration of the chemical by injection (mg/kg) or whole body exposure to different concentrations (ppm), no external toxicity symptoms were marked.
**Qualitative:** The location and size of micronuclei varied from cell to cell. In general one micronucleus per cell was recorded. Micronuclei were mostly dot shaped and range from 1/5 to 1/28th (*C. batrachus*) of the principal nucleus. Throughout the entire course of investigation both small and large size MN were observed in the treated individuals. Besides the induction of micronuclei, several other types of nuclear anomalies such as sickle shaped, thinning in mid region of nuclei and enucleated cells were also recorded.
**Quantitative:** In *C. batrachus* following i.p. treatment the frequency of micronuclei (MN) induced by two higher doses (0.3, 4.5mg/kg) increased significantly from respective controls (Table1) [*P<0.05,**P<0.01 (Student's t-test)]. Further, significant variations were also observed in dose response analysis (F=8.32;d.f.=36.3;**P<0.01). Moreover, a linear increase in the frequency of micronuclei with doses was marked (b=0.008;r=0.987;**P<0.01). However, no significant variation was observed in time response analysis [F=2.20;d.f.=36.2;P>0.05](ANOVA).

In dermal exposure experiment in *C. batrachus* the frequency of MN induced by two higher doses(25,35ppm) increased significantly from respective controls (Table 2), significant increase in the incidence of MN was observed in all treated groups after 48 and 72 hrs of exposure [*P<0.05; ** P<0.01 ; (Student's t-test)]. Further significant increase were also marked for different doses (F=8.44;d.f.=36.3;**P<0.01). However, no significant variation was observed in time response analysis (F=1.12; d.f. =36.2; **P<0.05) (ANOVA). Moreover, a linear increase in the frequency of micronuclei with dose was observed (b=0.063; r=0.983;*P<0.01)

## CONCLUSION

The research clearly demonstrated the clastogenic impact of copper sulfate on *Clarias batrachus*, raising significant concerns regarding potential health risks for humans and other aquatic organisms reliant on aquatic ecosystems and its agricultural use. Variation in micro nuclei rates across species and toxic agents suggests a correlation with toxin chemical kinetics and hemopoietic cycle speed. Genotoxic pollutants have been linked to gene mutations, posing a threat to future generations if left unchecked. While fish are often the first casualties, humans are also at risk. Peripheral blood sampling is an effective method for bio-monitoring projects, allowing multiple samples to be collected from the same individuals without sacrificing them. Further investigations using diverse test systems are necessary to reconcile contradictory findings regarding the chemical's effects .Conducting micro nuclei tests in fish erythrocytes at different intervals would enable monitoring changes in micronuclei frequencies over time.

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**Table 1: The occurrence of micro nucleated peripheral blood cells in *Clarias batrachus* fish injected intraperitoneally with copper sulfate $CuSO_4$, $5H_2O$**

| Dose(mg/kg) | Time(hrs) | No. ofMN | ‰ aberration ± S.E. | No. o fNA | ‰ aberration ±S.E |
|---|---|---|---|---|---|
| Control | 24 | 2 | 0.11±0.06 | 2 | 0.18±0.07 |
| | 48 | 2 | 0.12±0.07 | 2 | 0.17±0.08 |
| | 72 | 3 | 0.17±0.08 | 3 | 0.14±0.07 |
| 1.5 | 24 | 4 | 0.24±0.11* | 4 | 0.18±0.13 |
| | 48 | 5 | 0.31±0.08* | 5 | 0.25±0.11 |
| | 72 | 4 | 0.35±0.07* | 5 | 0.32±0.07 |
| 3 | 24 | 6 | 0.36±0.07** | 4 | 0.26±0.12 |
| | 48 | 6 | 0.38±0.08** | 5 | 0.32±0.06 |
| | 72 | 6 | 0.39±0.07* | 5 | 0.31±0.07 |
| 4.5 | 24 | 6 | 0.38±0.08** | 3 | 0.19±0.06 |
| | 48 | 7 | 0.44±0.07** | 5 | 0.32±0.07 |
| | 72 | 9 | 0.56±0.13** | 6 | 0.34±0.08 |

Results are mean‰ ± S.E of four fish.

Result is significantly different from the control at $^{*}P<0.05$, $^{**}P<0.01$(Student's t-test)16000 cells were scored for each point (4000/fish)

**Table2**: The occurrence of micro nucleated peripheral blood cells in *Clarias batrachus* fish exposed to water contaminated with $CuSO_4$, $5H_2O$.

| Dose(ppm) | Time(hrs) | No. of MN | ‰ aberration ± S.E. | No. of NA | ‰ aberration ± S.E |
|---|---|---|---|---|---|
| Control | 24 | 2 | 0.11±0.06 | 2 | 0.18±0.08 |
| | 48 | 3 | 0.11±0.06 | 2 | 0.18±0.07 |
| | 72 | 3 | 0.15±0.08 | 3 | 0.14±0.06 |
| 15 | 24 | 4 | 0.24±0.11* | 4 | 0.23±0.09 |
| | 48 | 6 | 0.32±0.07* | 5 | 0.32±0.06 |
| | 72 | 6 | 0.32±0.08* | 5 | 0.32±0.08 |
| 25 | 24 | 5 | 0.30±0.09** | 5 | 0.32±0.07 |
| | 48 | 6 | 0.37±0.08** | 5 | 0.38±0.07 |
| | 72 | 8 | 0.44±0.11* | 6 | 0.38±0.07 |
| 35 | 24 | 4 | 0.32±0.07** | 5 | 0.32±0.09 |
| | 48 | 6 | 0.41±0.08** | 5 | 0.36±0.08 |
| | 72 | 8 | 0.54±0.13** | 7 | 0.37±0.08 |

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**RESEARCH ARTICLE**

# Assessment of Sleep Quality among the Patients Attending the Gastroenterology Department

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## ABSTRACT

To evaluate the quality of sleep among the patients with various gastrointestinal disorders. Sleep quality was assessed by using Pittsburg Sleep Quality Index (PSQI) scale among the patients with gastro intestinal disorders. Patients who were willing to participate in the study with age above 18 years, who were diagnosed with gastrointestinal disorders like inflammatory bowel syndrome (IBS), inflammatory bowel disease (IBD), peptic ulcer disease (PUD), gastro esophageal reflux disease (GERD), Chron's disease (CD), functional dyspepsia (FD), acute & chronic gastritis, gastro-paresis, gastric and duodenal erythemias were included in the study. Patients with chronic debilitating diseases such as cancer, hepatic, renal & cardiac diseases, past history of psychiatric illness and sleep disorders were excluded from the study. The sleep component scores are added to yield a total score ranging from 0 to 21 with the higher total score (referred to as global score) indication of poor sleep quality or worse sleep quality. A global PSQI score over 5 indicates poor sleep relative to clinical and laboratory measures, and higher scores indicate poorer sleep quality. A total of 193 gastrointestinal disorder patients were participated in the study and among them 109 (56.5%) were found to be males and 84 (43.5%) were found to be females. In this study, most of the study participants were observed in the age group 31-40 years (26.9%) followed by 21-30 years (23.8%). Most of the study participants were observed with erosive gastritis (20.7%) followed by GERD (19.2%). Majority of the study

participants were suffering from various GI related issues with duration of more than 1-5 years (40.9%). In this study, most of the study participants were observed with poor sleep quality (81.9%) followed by good sleep quality (18.1%). In this study, about 81.9% of the study participants were observed with poor sleep quality and only 18.1% of the study participants were observed with good sleep quality. Erosive gastritis, GERD and gastric & duodenal ulcers were the most commonly observed gastrointestinal disorders. Clinical pharmacists in association with the other health care professionals should take the responsibility in creating the awareness regarding the good sleep quality index by taking the necessary precautions in dealing the various GI disorders thereby improving the sleep quality index which may impact the health related quality of life of the patients.

**Keywords:** Clinical Pharmacist, Gastrointestinal disorders, Pittsburg Sleep Quality Index.

## INTRODUCTION

Sleep disturbances includes a numerous types of disorders such as narcolepsy, sleep apnea, insomnia, hypersomnia, circadian rhythm sleep-wake disorders & parasomnia, cataplexy and movement problems associated with sleep [1,2]. A lot of individuals struggle with sleep disorders, among them insomnia is one of the most prevalent disorder [3]. The American Psychiatric Association states that, 10-20% of patients complain to their primary care physician about their severe sleep related problems [4]. In another previous research, about 16.6% of patients with 50 years of age or older, nocturnal sleep issues were reported [5]. The incidence of sleep disruptions increases with age. In geriatrics, most of them were observed to be with sleep related illness and are not satisfied with their sleep quality [6]. Various functional changes in the gastrointestinal (GI) tract have been reported during the sleep or due to disturbed sleep. These changes include increased transient relaxations of the lower esophageal sphincter which aggravates gastro-esophageal reflux [7] & motor abnormalities in the upper GI tract and small bowel. Trouble sleeping has also been linked to visceral hypersensitivity [8-10].

Patients suffering from various gastrointestinal illnesses such as irritable bowel syndrome (IBS), functional dyspepsia (FD), inflammatory bowel disease (IBD) and gastro esophageal reflux disease (GERD) are most frequently observed with poor quality of sleep [11-14]. According to community-based cohort studies, poor sleep quality is stated in 68% of people with GERD, 77% of people with active IBD and 49% of people with inactive IBD [15, 16]. According to other studies, 30–60% of IBS patients and 68% of FD patients were also reported with trouble sleeping, feeling tired throughout the day or both [17-21]. The Pittsburgh Sleep Quality Index, a psychological or behavioral concept is considered as one of the most reliable assessment for evaluating sleep quality and its patterns. PSQI has been used to measure poor sleep quality in the majority of research that is currently quality of life of GI patients have been demonstrated to be significantly impacted by poor sleep quality [20, 22]. Exhaustion and sleeping during the daytime are the most annoying extra-intestinal symptoms that are linked to inflammatory bowel syndrome (IBS) and inflammatory bowel disease (IBD) [17, 23]. In some of the studies along with inadequate sleep or poor sleep quality, the GI symptoms are also reported the following day [24, 25] which can also be associated with chronic pain disorders such as elevated hyperalgesia and inflammation [26-29].

In the patients with gastrointestinal disorders, self reported sleep problems were not clearly defined whereas the majority of sleep related research was focused on the IBS and functional problems. So, there is a necessity to know how sleep features vary in different GI disorders. Therefore, in this study we attempted to evaluate the quality of sleep among the patients with various gastrointestinal disorders.

## MATERIALS AND METHODS

This is a prospective study conducted for a period of 6 months with a study-population of 193 members, at Sri Hasitha Gastro and Diabetes hospital, Rajahmundry. Data was collected after getting the ethical committee approval

and also the approval from the above mentioned hospital there by strictly considering the inclusion and exclusion criteria of the study. In patients with gastro intestinal disorders, sleep quality was assessed by using Pittsburg Sleep Quality Index (PSQI) scale. The patients visited to the hospital were enrolled in the study by considering the study criteria after taking their consent to participate in the study. Patients who were willing to participate in the study with an age of above 18 years, who were diagnosed with gastrointestinal disorders like inflammatory bowel syndrome (IBS), inflammatory bowel disease (IBD), peptic ulcer disease (PUD), gastro esophageal reflux disease (GERD), Chron's disease (CD), functional dyspepsia (FD), acute & chronic gastritis, gastro-paresis, gastric and duodenal erythemias were included in the study. Patients with chronic debilitating diseases such as cancer, hepatic, renal & cardiac diseases, past history of psychiatric illness and sleep disorders were excluded from the study. In this study, Pittsburg Sleep Quality Index Questionnaire was used to assess the sleep quality. There are seven components and each of the sleep components yields a score rangingfrom 0 to 3, with 3 indicating the greatest dysfunction. The total score is obtained by adding the sleep component scores, which can range between 0-21. Global score of PSQI greater than 5 represents the poor sleep quality related to the clinical or the laboratory parameters [30].

## RESULTS AND DISCUSSION

A total of 193 gastrointestinal disorder patients were participated in the study and among them 109 (56.5%) were found to be males and 84 (43.5%) were found to be females. In this study, most of the study participants were observed in the age group 31-40 years (26.9%) followed by 21-30 years (23.8%). Majority of the study participants were found to be married (85%) followed by unmarried (15%). In this study, most of the study participants were non-smokers (75.1%) and non-alcoholics (79.3%). Majority of the study participants were educated and more than half of the study participants were observed with full time employment (55.5%).

Most of the study participants were observed with erosive gastritis (20.7%) followed by GERD (19.2%). Majority of the study participants were suffering from various GI related issues with duration of more than 1-5 years (40.9%). In this study, most of the study participants were observed with poor sleep quality (81.9%) followed by good sleep quality (18.1%). Categorization of the study participants based on the sleep quality index among various gastro intestinal disorders was tabulated in table 3.

## CONCLUSION

In this study, about 81.9% of the study participants were observed with poor sleep quality and only 18.1% of the study participants were observed with good sleep quality. Erosive gastritis, GERD and gastric & duodenal ulcers were the most commonly observed gastrointestinal disorders. Clinical pharmacists in association with the other health care professionals should take the responsibility in creating the awareness regarding the good sleep quality index by taking thenecessary precautions in dealing the various GI disorders thereby improving the sleep quality index which may impact the health related quality of life of the patients.

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**Table 1: Categorization of the study participants based on the patient characteristics**

| Characteristics | | Male (%) | Female (%) | Total (%) |
|---|---|---|---|---|
| **Age** | 21-30 | 28 (25.7) | 18 (21.4) | 46 (23.8) |
| | 31-40 | 24 (22.0) | 28 (33.4) | 52 (26.9) |
| | 41-50 | 23 (21.1) | 18 (21.4) | 41 (21.2) |
| | 51-60 | 11 (10.1) | 8 (9.5) | 19 (9.9) |
| | 61-70 | 11 (10.1) | 10 (11.9) | 21 (10.9) |
| | 71-80 | 9 (8.2) | 2 (2.4) | 11 (5.7) |
| | 81-90 | 3 (2.8) | 0 (0) | 3 (1.6) |
| **Marital Status** | Married | 90 (82.6) | 74 (88.1) | 164 (85) |
| | Unmarried | 19 (17.4) | 10 (11.9) | 29 (15) |
| **Smoking** | Non-Smokers | 62 (56.9) | 83 (98.8) | 145 (75.1) |
| | Smokers | 47 (43.1) | 1 (1.2) | 48 (24.9) |
| **Alcoholism** | Non-Alcoholics | 69 (63.3) | 84 (100) | 153 (79.3) |
| | Alcoholics | 40 (36.7) | 0 (0) | 40 (20.7) |
| **Education** | Primary | 31 (28.5) | 21 (25) | 52 (26.9) |
| | Secondary | 26 (23.8) | 23 (27.4) | 49 (25.4) |
| | Tertiary | 39 (35.8) | 29 (34.5) | 68 (35.2) |
| | Uneducated | 13 (11.9) | 11 (13.1) | 24 (12.5) |
| **Employment** | Full Time | 84 (77.1) | 23 (27.4) | 107 (55.5) |
| | Part Time | 9 (8.3) | 3 (3.6) | 12 (6.2) |
| | Retired | 1 (0.9) | 1 (1.2) | 2 (1.0) |
| | Students | 2 (1.8) | 2 (2.4) | 4 (2.1) |
| | Long term sick leave | 8 (7.3) | 0 (0) | 8 (4.1) |
| | Unemployed/Home makers | 5 (4.6) | 55 (65.4) | 60 (31.1) |

**Table 2: Categorization of the study participants based on the disease characteristics**

| Characteristics | | Male (%) | Female (%) | Total (%) |
|---|---|---|---|---|
| **GI Disorders** | Chronic Gastritis | 9 (8.3) | 7 (8.3) | 16 (8.3) |
| | Chron's Disease | 2 (1.8) | 2 (2.4) | 4 (2.0) |
| | Erosive Gastritis | 24 (22.0) | 16 (19.0) | 40 (20.7) |
| | Functional Dyspepsia | 9 (8.3) | 9 (10.7) | 18 (9.3) |
| | Gastric & Duodenal Ulcers | 15 (13.8) | 7 (8.4) | 22 (11.4) |
| | Gastroparesis | 1 (0.9) | 0 (0) | 1 (0.5) |
| | GERD | 21 (19.3) | 16 (19.0) | 37 (19.2) |
| | GERD, Hiatus Hernia | 10 (9.2) | 2 (2.4) | 12 (6.3) |
| | Inflammatory Bowel Disease | 2 (1.8) | 6 (7.2) | 8 (4.1) |
| | Inflammatory Bowel Syndrome | 2 (1.8) | 8 (9.5) | 10 (5.2) |
| | Peptic Ulcer Disease | 9 (8.3) | 3 (3.6) | 12 (6.3) |
| | Ulcerative Colitis | 5 (4.5) | 8 (9.5) | 13 (6.7) |
| **Disease duration (in years)** | <1 years | 47 (43.1) | 30 (35.7) | 77 (39.9) |
| | 1-5 years | 39 (35.8) | 40 (47.6) | 79 (40.9) |

| | | | | |
|---|---|---|---|---|
| | >5 years | 23 (21.1) | 14 (16.7) | 37 (19.2) |
| **Sleep Quality Index** | Poor (PSQI≥5) | 87 (79.8) | 71 (84.5) | 158 (81.9) |
| | Good (PSQI<5) | 22 (20.2) | 13 (15.5) | 35 (18.1) |

**Table 3: Categorization of the study participants based on the sleep quality index in various gastro intestinal disorders**

| GI Disorder | PSQI≥5 (%) | PSQI<5 (%) | TOTAL (%) |
|---|---|---|---|
| Chronic Gastritis | 13 (81.3) | 3 (18.7) | 16 (100) |
| Chron's Disease | 3 (75) | 1 (25) | 4 (100) |
| Erosive Gastritis | 30 (75) | 10 (25) | 40 (100) |
| Functional Dyspepsia | 14 (77.8) | 4 (22.2) | 18 (100) |
| Gastric & Duodenal Ulcers | 16 (72.7) | 6 (27.3) | 22 (100) |
| Gastroparesis | 1 (100) | 0 (0) | 1 (100) |
| GERD | 31 (83.8) | 6 (16.2) | 37 (100) |
| GERD, Hiatus Hernia | 9 (75) | 3 (25) | 12 (100) |
| Inflammatory Bowel Disease | 7 (87.5) | 1 (12.5) | 8 (100) |
| Inflammatory Bowel Syndrome | 9 (90) | 1 (10) | 10 (100) |
| Peptic Ulcer Disease | 12 (100) | 0 (0) | 12 (100) |
| Ulcerative Colitis | 13 (100) | 0 (0) | 13 (100) |
| Total | 158 (81.9) | 35 (18.1) | 193 (100) |

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**RESEARCH ARTICLE**

# A Spectacular Appraisal on Megasaanthi Chooranam – A Cure to Ease Pain

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## ABSTRACT

Siddha is one of the treatment modality in Indian traditional system. Siddha is not only a system of medicine, but it also has tight bond with the day-to-day life of the population(Tamil people). Nowadays, the consumption of siddha medicine is drastically increased due to its efficacy and nil or less side effects. Megasaanthi Chooranam is mentioned in one of the ancient Siddha text, *Kannusamy pillai's Chikicha rathna deepam.* It is indicated for soolai, megavaayu, piduppu, kiranthi, megapadai, megaooral[1] etc. The term sooolai is referred to pain. Among these indications, authors of this manuscript explains about the management of pain through the above said drug. The Scientific community are not ready to accept the medicines due to lack of proper scientific validation. So, authors of this manuscript decided to prove the efficacy of the drug Megasaanthi Chooranam through review process in the aspect of pain management. This review article deals about the ethnopharmacological profile and phyconstituents of ingredients of Megasaanthi Chooranam.

**Key Words:** *Megasaanthi Chooranam, Chikicha rathna deepam, Siddha medicine.*

# INTRODUCTION

Pain is characterised as an unpleasant and emotional feeling that may or may not be accompanied by actual tissue damage. The pain sensation is described in many ways like sharp, pricking, electric, dull ache, shooting, cutting, stabbing etc.

**Classification of pain**
Depending on the duration of pain, it is classified into

**Acute pain:**
Acute pain is a sharp, brief-lasting ache with a clear cause. Before affecting nearby locations, it frequently first becomes localised in a limited area. Typically, drugs are used to treat it.

**Chronic pain**
Chronic pain is a type of persistent, varying-intensity pain. It continues for a longer time. Chronic pain is challenging to treat and requires specialised medical attention.
However, depending on the location of the discomfort, it may classified into

**Visceral pain**
Pain from viscera is unpleasant. It is poorly localized. The causes of visceral pain include ischemia, chemical stimuli, spasm of hollow organs and over distension of hollow organs.

**Referred pain**
Referred pain is pain that is felt at a location close to or distant from its original location. Referrals to other locations are made for the severe pain and some visceral pain. But there is no referral for the minor discomfort.
The examples of referred pain includes,

- The inside portion of the left arm and left shoulder are also affected by cardiac discomfort.
- Pain in ovary is referred to umbilicus
- Pain from testis is felt in abdomen.
- Pain in diaphragm is radiates to right shoulder.
- Pain in gall bladder is referred to epigastric region.
- Renal pain is referred to loin.

The neurotransmitters involved in pain sensation include Glutamate and substance P which were secreted by the pain nerve endings. The afferent fibers which transmit impulses of fast pain secrete glutamate. C type fibers secretes substance P that transmit impulses of slow pain secrete substance P. As this review article deals with pain management it is necessary to understand about the analgesia system. Analgesia system means the pain control system. Analgesic system in brain provides a short term relief from pain. The analgesia system has got its own pathway through which it blocks the synaptic transmission of pain sensation in spinal cord and thus attenuates the experience of pain. In fact analgesic drugs such as opioids act through this system and provide a controlled pain relief[3]. This review article is all about the phytoconstituents of the drug and pharmacological activities corresponding to the indication of pain management .

**INGREDIENTS OF MEGASAANTHI CHOORANAM**

1. PARANGICHAKKAI (*Smilax china.Linn)*
2. AMUKKARA KIZHANGU (*Withania somnifera.Linn)*
3. VELLARUGU (*Enicostemma axillare.Linn)*
4. THUMRASHTAM *(Alpinia galangal.Linn)*

5. SANGAM PATTAI (*Azima tetracantha.Linn)*
6. MILAKARANAI PATTAI (*Toddalia asiatica.Linn)*
7. CHITHIRAMOOLA PATTAI (*Plumbago indica.Linn)*
8. CHUKKU (*Zingiber officinale.Linn)*
9. THIPPILI (*Piper longum.Linn)*
10. THANDRIKKAI (*Terminalia bellirica.Linn)*
11. KADUKKAI (*Terminalia chebula.Linn)*
12. KANDUPARANGI (*Clerodendrum serratum.Linn)*
13. SOMBU (*Pimpinella anisum.Linn)*
14. ELAM (*Elettaria cardamomum.Linn)*
15. VAIVILANGAM (*Celastrus paniculatus.Linn)*
16. ANNASIPPU (*Ilicium verum.Linn)*
17. CHEVVIYAM ( *Piper nigrum.Linn)*
18. ATHIMADURAM (*Glycyrrhiza glabra.Linn)*
19. KOSHTAM (*Costus speciosus.Linn)*
20. MODI (*Piper longum.Linn)*

**PHYTOCONSTITUENTS OF MEGASAANTHI CHOORANAM**

**Parangichakkai (*Smilax china*)**

Beta-sitosterol, Caffeic acid, catechin, daucosterin, daucosterol, engeletin, epicatechin,
friedelin, heloniosides, hydroxyflavan, isoengeletin, naringenin, piceid, quercetin, resin, resveratrol, rutin, saponin, scirpusin, seiboldogenin, smilacin, smilasides, tannin, taxifolin, trihydroxystibene, vanillic acid, flavonoids and stilbenes[4] .

**Amukkara kizhangu (*Withania somnifera*)**

The powdered extract derived from Withania somnifera's root reveals the existence of carbohydrates, starch, tannins, saponins, glycosides, phenolic compounds, and alkaloids. Aqueous extract contains amino acids, flavonoids and saponins while ethanolic extracts indicate the presence of saponin, alkaloids, phenolics, glycosides, starch, terpenoids and flavonoids[5] .

**Vellarugu (*Enicostemma axillare*)**

The petroleum ether extracts contains glycosides, alkaloids and flavanoids. The chloroform ether extract contains glycosides and alkaloids. The methanol extract contains tannins, alkaloids, glycosides and flavanoids[6] .

**Thumrashtam (*Alpinia galangal*)**

Flavonoid, 1′S-1′-acetoxychavicol acetate (ACE), phenylpropanoids and phydroxybenzaldehyde (1′S-1′-acetoxychavicol acetate and 1′S-1′- acetoxyeuginol acetate), acetoxycineoles (trans and cis)-2- and 3-acetoxy- 1, 1, 8-cineoles, 1′-acetoxychavicol acetate (galangal acetate), β-Sitosterol diglucoside (AG-7) and β-sitsteryl Arabinoside (AG-8), hydroxy-1,8-cineole glucopyranosides, (1R, 2R, 4S) –and (1S, 2S, 4R)-trans-2-hydroxy-1, 8-cineole β-D-glucopyranoside, and (1R, 3S, 4S)-trans-3-hydroxy-1, 8-cineole β-D-glucopyranoside[7] .

**Sangan (*Azima tetracantha*)**

The plant contains alkaloids, flavonoids, sterols, terpenoids, volatile oil and fatty acids, saponins. Dimeric piperidine alkaloids azimine, azcarpine and carpine are present in all plant parts[8].

**Milakaranai (*Toddalia asiatica*)**

Stigmasterol, β-sitosterol, lyoniresinol, dl-syringaresinol, oxyterihhanine, integriamide, peucedanol methylether, dotriacontanol, hexacosanoic acid, heptacosanoic acid, euscaphic acid, arjunic acid, benzoic acid, D-mannitol, and rhamnose have been extracted and identified from different anatomical components of the plant.

**Chithiramoolam (*Plumbago indica*)**

P. indica is rich in different types of alkaloids, flavonoids, saponins, glycosides and tannins. methyl 16-methylheptadecanoate, sec-Butyl isobutyl sulfide, 4-Amino-6-hydroxypyrimidine, Pyridine,4-(1,1-dimethylethyl), Succinic acid,di(3,5- dimethylcyclohexyl) ester, 8 Octadecenoic acid,methyl ester, 4-Hydroxypyridine 1-oxide, Cyclohexane,1,4-dimethyl-2-octadecyl, 2,Thiophenecarboxylic acid, 3,5-dimethylcyclohexyl ester and Hexadecanoic acid, methyl ester were detected but only methyl 16-methylheptadecanoate, 8-Octadecenoic acid, methyl ester and Hexadecanoic acid,methyl ester[10,11].

**Chukku (Dried rhizome of *Zingiber officinale*)**

Gingerol, Shogoal, Zingerone, Gingerine, Gingeberol, Essential oil-Camphene, phellandrene, cineol, zingiberene and borneol, Resin and starch. Dried ginger has been a traditional herbal remedy for thousands of years in Asian, Indian, Arabic medicine because it contains a higher concentration of two active compounds Shagoal and The concentration of gingerol in dried ginger is approximately threefold higher than that in fresh ginger. Shagoal, characterized by its pungency, serves as ginger's potent component, known for its robust anti-cough properties. Gingerol, among the various active constituents within ginger, imparts the spice with its distinctive varieties of piquancy. [12,13].

**Thippili (Immature berries of *Piper longum*)**

Alkaloids include piperine, piperlonguminine, piperlongumine and methyl-3,4,5-trimethoxycinnamate. The fruit part contains volatile oil (1%), protein, starch, alkaloids, saponins, carbohydrates and amygdalin, a waxy alkaloid N-isobutyldeca-trans-2-trans-4-dienamide, alkaloids piperine, calcium, phosphorus, iron and a terpenoid substance. Lignans and esters such as sesamin, pulvatilol, furgesin, Z-12-octadeconic –glycerol-monoester, tridecyl-dihydro-p-coumarate were also isolated from the fruit part of the plant. The root of the plant contains piperlongumine or piplartinine, piperine and dihydrostigmasterolasarinine, pellitorine, refractomide A, brachystine, pipercide, piperderidine, piperundecalidine, iperonaline, methyl piperine, tetrahydropiperlongumine, dehydropipernonaline, piperidine, trimethoxy cinnamoyl – piperidine and piperlongumine. Other chemical components present are sesamine, dihydro stigmasterol, piperacide, piperundecalidine, pipernonaline, dieudesmin and sitosterol[14].

**Thandrikkai (*Terminalia bellirica*)**

Flavone includes 7- hydroxy 3′,4′ (methylenedioxy)flavone and luteoline. Steroids-β-Siteserol. Lignans include Termilignan, thannilignan, anolignan B. Tannins include gallic acid, ellagic acid, methyl gallate, ethyl gallate (Phenyllemblin), chebulaginic acid, chebulagic acid and hexahydroxydiphenic acid ester. Glycosides – Fructose, sucrose, galactose, D-glucose, mannose and rhamnose. Terpenoids like Belleric acid, chebulagic acid and arjungenin. Saponin- bellericoside and bellericanin. Cardenolide includes Cannogenol 3-O-β-galactopyranosyl-(1→4)-O-α-L-rhamopyranoside. Flavonol aglycones – Quercetine and kampferol. Flavonol glycosides – quercetin-3-O-[6′′-α-L-rhamnopyranosyl]-(1→6)-β-D-glucopyranoside (rutin), quercetin -3-O-α-L-rhamnopyranoside, quercetin -3-O-β-D-glucopyranoside and kaempferol-3-O- β-D- glucopyranoside. Glycerides of fatty acids include palmitooleolinolein, stereo-oleolinolein, palmitodiolein, stereodiolein, dioleolinolein, and triolein[15].

**Kadukkai (*Terminalia chebula*)**

Glycosides include triterpines arjunglucoside I, arjungenin and chebulosides I and II. Coumarin include chebulin. Phenolic components present are ellagic acid, 2,4-chebulyl-β-D-glucopyranose, chebulinic acid, gallic acid, ethyl gallate, punicalagin, terflavin A, terchebin, luteolin and tannic acid. Chebulinic acid is a phenolic acid isolated from the ripe fruits. Luteic acid is isolated from the bark. Fruits also contain terflavin B, tannin and chebulinic acid. The other phytoconstituents present in fruit are tannins, anthraquinones, chebulinic acid, chebulagic acid, chebulic acid, ellagic acid and gallic acid. The other components include corilegin, β-D-glucogallin, glucose and sorbitol. Polyphenolic compounds triterpene glycosides, terchebulin, punicalagin, terflavin A, flavonoids, reducing sugars and starch are also present in the fruit. Terpenene glycosides, arjungenin and arjunglucoside-I. 18 amino acids and a small quantity of phosphoric, succinic, syringic and quinic acids[16].

**Kanduparangi (*Clerodendrum serratum*)**
In root the following phytoconstituents are present. Saponins, D-mannitol, stigmasterol, oleanolic acid, queretaroic acid, serratagenic acid, sitoserol, clerosterol is found as 5, 25-stigmastadien-3β o, clerodone as 3β-hydroxyl-lupan 12-one, B-sitosterol, lupeol, a steroidal glycoside, phytosterols, ferulic acid, arabinose, scutellarcin, baicalein, serration and ursolic acid. Leaf contains catchin, α-spinosterol, luteoline, polyphonolics, diterpin-clerodin, ethycholesta-5, 24 25 –trine 3β- o hispidulin and 7-o- gluconoids of hispidulin and cruteuarein. The other chemical constituents of clerodendrm serratum are carbohydrates, phenolics, flavonoids, terpenoids and steroids[17].

**Sombu (*Pimpinella anisum*)**
The major compounds were anethole (~90%), γ-hima-chalene (2–4%), p-anisaldehyde (<1%), methylchavicol (0.9–1.5%), cis-pseudoisoeugenyl 2-methylbutyrate (~3%), and trans-pseudoisoeugenyl 2-methylbutyrate (~1.3%). Neophytadiene – a terpene hydrocarbon was isolated from aniseed[18].

**Elam (*Elettaria cardamomum*)**
Flavonoids, alkaloids, tannins, aromatic compounds, saponins, phytosterols, and phenolic compounds, terpenoids. In methanol extract carbohydrates, phenols, flavonoids, saponins, glycosides, steroids and alkaloids are present[19].

**Vaivilangam (*Embelia ribes*)**
The ripe fruit contains embelin. Berries contains embelin, volatile oil, fixed oil, resin, tannin, christembine, phenolic acids such as caffeic acid, vanillic acid, chororogenic acid, cinnamic acid and o-cumaric acid,. Phenolic acids include caffeic acid, vanillic acid, chororogenic acid, cinnamic acid and o-cuma. The seeds contains embelinol, embeliaribyl ester and embeliol. The seeds also contains Cr, K, Ca, Cu, Zn and Mn. The alcoholic extract of embelia ribes fruit powder contains carbohydrates, gum, mucilage, amino acid, steroids, glycoside, anthraquinone glycoside, flavonoids, saponin glycoside, alkaloids, tannins and phenolic compounds. The aqueous extract of embelia ribes fruit powder contains carbohydrates, gum, amino acid, glycoside, anthraquinone glycoside, flavonoids, saponin glycoside, alkaloids, tannins and phenolic compounds[20].

Annasippu (*Ilicium verum*)
Alkaloids, flavonoids, saponins, steroids, tannins, protein and amino acids are present. It also contains moisture (10.22%), ash (2.87%), protein (4.25%), fat (7.65%), carbohydrates (75.01%), crude fiber (11.12%) and metabolic energy (385.89 Kcal/100g). The mineral nutrients present are calcium, magnesium, phosphorus, sulphur, potassium, sodium and chlorine. Trace elements include iron, manganese, copper, molybdenum, selenium, chromium, tin, zinc, nickel, fluorine, and cobalt [21].

**Chevviyam (Root of *Piper nigrum*)**
It contains volatile alkaloid piperine, piperidine, piperidin, volatile oil, chavicin, starch, lignin. Sabinene (15-25%), Limonene (5-20%), Caryophyllene (10-15%), β-pinene (10-12%), α-pinene (8-12%), acid amides [22].

**Athimaduram (*Glycyrrhiza glabra*)**
Alkaloids, glycosides, triterpenes, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, asparagins, bitters, phenolics, saponins, tannins, terpenes, anthraquinones, essential oils, fat, female hormone oestrogen, gums, mucilage, protein, resins, starches, sterols and volatile oils[23,24].

**Koshtam (*Costus speciosus*)**
The roots of this plant also contain prosapogenins A and B of dioscin, dioscin, gracillin, protodioscin and methyl protodioscin. Other components identified were 31-norcycloartanone, cyloartanol, cycloartenolandcycloalaudenol. The petroleum ether extracts of the stems and roots of C.*speciosus*, diosgenin and sitosterol were isolated[25].

**Modi (Root of *Piper longum*)**

The root part of the plant contains piperlongumine or piplartinine, piperine and dihydrostigmasterolasarinine, pellitorine, refractomide A, brachystine, pipercide, piperderidine, piperundecalidine, iperonaline, methyl piperine, terahydropiperlongumine, dehydropipernonaline piperidine, trimethoxy cinnamoyl-piperidine and piperlongumine. The other alkaloids components present are asarinine, pellitorine, refractomide A, brachystine, pipercide, piperderidine, piperundecalidine, iperonaline, methyl piperine, terahydropiperlongumine, dehydropipernonaline, piperidine, trimethoxy cinnamoyl piperidine and piperlongumine[14].

**PHARMACOLOGICAL ACTIVITIES OF MEGASAANTHI CHOORANAM**

**Parangichakkai (Smilax china)**

**Anti-inflammatory activity**

Shu X.S. et al., studied that the Smilax chinensis has anti-inflammatory activity that is evident on rats. They also investigated Smilax chinensis for anti-inflammatory effects in rats with egg albumin-induced edema in mice using hot-plate test and acetic acid-induced abdominal constriction test, respectively. The data indicates that the extract inhibits both COX activity inhibitors and COX expression. Due to these pharmacological effects, this plant extract can be used to treat a number of inflammatory conditions [26].

**Amukkara kizhangu (*Withania somnifera*)**

**Anti-inflammatory activity**

Uddin et al., Al-Hindawi et al., studied that Withania somnifera reported to possess anti-inflammatory property in different pharmacological animal models of inflammation such as carrageenan-induced inflammation, cotton pellet granuloma and adjuvant-induced arthritis. It inhibited the granuloma formation in cotton-pellet implantation in rats similar to that of hydrocortisone sodium succinate [27,28].

**Analgesic activity**

Rasool M et al., studied that *Withania somnifera* extract also shows potent analgesic effect by retarding amplification and propagation of the inflammatory response in monosodium urate crystal-induced experimental rat models, without causing any gastric damage compared to indomethacin, a non-steroidal anti-inflammatory drug [29].

**Vellarugu (*Enicostemma axillare*)**

**Anti-inflammatory activity**

Mizushima et al., and Sakat et al., studied the anti-inflammatory activity of *Enicostemma axillare* by using inhibition of albumin denaturation technique [30,31]. G.Leelaprakash et al., revealed that results indicate that the methanol extracts of *Enicostemma axillare* possess anti-inflammatory properties. These actions can likely be attributed to the robust presence of polyphenolic compounds like alkaloids, flavonoids, tannins, steroids, and phenols. The extract fractions function as free radical inhibitors or scavengers, potentially acting as primary oxidants, thereby inhibiting heat-induced albumin denaturation, proteinase activity, and stabilizing the membrane of Red Blood Cells. [32].

**Thumrashtam (*Alpinia galangal*)**

**Analgesic and anti-inflammatory activity**

Nagashekhar M et al., studied the analgesic activity and anti-inflammatory of the topical. The process involved in creating a methanolic extract from Alpinia galangal wild was followed by assessing its anti-inflammatory properties using carrageenan-induced edema tests in rats as well as a formalin test. Piroxicam gel and methyl salicylate ointment were studied as positive controls for anti-inflammatory and analgesic activities, respectively [33]. Jaju S et al., reported that the methanolic and phenolic extract of the rhizome of alpinia galanga exhibit antidiabetic and anti-inflammatory properties [34].

**Sangan (*Azima tetracantha*)**

**Analgesic Activity**

Nandgude et al., screened the benzene, chloroform and aqueous extracts of leaves of A.tetracantha for analgesic activity of in using a hot plate method. The extracts displayed marked analgesic potential with significant analgesic activity at a dose of 100 mg/kg body weight. Marked analgesic activity observed at 30 min after extract administration which was near equivalent to that of morphine sulfate [35].

**Anti - inflammatory activity**

Sridharan et al., screened for ethanolic extract obtained from whole plant of A. tetracantha for anti-inflammatory activity by carrageenan-induced paw edema method. The extract at 250–500 mg/kg dose suppressed the paw edema significantly at 3–4 hr [36].

**Milakaranai (*Toddalia asiatica*)**

**Anti - inflammatory activity**

Kavimani et al., studied the volatile oil of the leaves obtained by steam distillation on the exudative and proliferative phases of the inflammatory reactions using carrageenin induced paw edema and cotton pellets granuloma in male albino rats. The anti-inflammatory effect of the oil was attributed to inhibition of histamine, kinin and prostaglandin. It also showed effect in cotton pellets granuloma due to suppression of the proliferation phase of inflammation which involves the proliferation of macrophages, neutrophils and fibroblasts. The oil was also found to be very safe as it did not show any toxic manifestations even upon continuous administration for 10 days [37].

Analgesic activity

Xiao-Yan Hao et al., studied the crude alkaloids for their pharmacological actions and toxicity in rats and found that they had analgesic activity showing decrease in body distortions in rats. It was observed that the alkaloids did not cause any injury to the liver even after long term administration[38].

**Chithiramoolam (*Plumbago indica*)**

**Analgesic and anti – inflammatory activity**

Ittiyavirah SP et al., evaluated analgesic and anti-inflammatory activities by using male wistar rats and mice in aqueous extracts of leaves of *P. capensis* and *P. indica.* A significant inhibition in the Carrageenan induced paw oedema at the dose of 300 mg/kg body weight was observed in comparison to the control group which was given standard drug Indomethacin[39].

**Chukku (Dried rhizome of *Zingiber officinale*)**

**Anti Inflammatory Activity**

Vendruscolo et al., conducted an experimental trial on male Swiss mice and male Wistar rats for the evaluation of anti-inflammatory effect. In this study they found that ginger essential oil (GEO) showed significant anti-inflammatory activity[40].

**Thippili (Immature berries of *Piper longum*)**

**Anti Inflammatory Activity**

Kumar S et al., and Choudhary GP et al., reported that the fruit extract of *Piper longum* were to possess anti-inflammatory activity in carageanan rat paw edema[41,42]. Stohr JR et al., reported that the piper extract and piperine possess inhibitory activities on prostaglandin and leukotrienes Cox-1 inhibitory effect and thus exhibit anti inflammatory activity[43].

**Thandrikkai (*Terminalia bellerica*)**

**Analgesic Activity**

Sharma US et al., investigated the analgesic activity of ethanolic and aqueous extract of *Terminalia bellerica* fruits in acetic acid induced writhing, Eddy's hot plate method and brener's yeast induced fever models in mice and rats.

There was a significant inhibition of elevated body temperature by both extracts as compared to the corresponding control group. Hence, results indicate that the ethanolic and aqueous extract possessed significant analgesic and antipyretic activities[44].

**Kadukkai (Terminalia chebula)**

**Anti-Inflammatory Activity**

Pratibha N et al., studied the aqueous extract of dried fruit of *T.chebula* and it showed anti-inflammatory by inhibiting inducible nitric oxide synthesis. *Terminalia chebula* in a polyherbal formulation (Aller-7) exhibited a dose dependent anti inflammatory effect in rats[45].

**Kanduparangi (*Clerodendrum serratum*)**

**Anti-inflammatory activity**

Narayanan et al., studied that the alcoholic extract of roots of *Clerodendrum serratum* was administered to Albino rats at the concentration of 50, 100, 200 mg/kg per orally to monitor ant- inflammatory activity by carrageenan induced paw edema and cotton pellet implantation methods. In both the acute and chronic models, a standard anti-inflammatory agent, phenylbutazone (administered orally at a dose of 100 mg/kg), served as a reference for comparison. *Clerodendrum serratum* demonstrated a robust anti-inflammatory impact, as evidenced by a noteworthy decrease in paw edema and cotton-pellet granuloma formation. Nevertheless, this effect was comparatively weaker when juxtaposed with the efficacy of phenylbutazone. [46].

**Analgesic activity**

Saha et al., evaluated the analgesic effect of the ethanolic extract of leaves of Clerodendrum serratum .Linn at the dose of 200 and 500 mg/kg by tail flick method and acetic acid induced writhing test in Wistar rats for seven days orally and standard group rats were administered diclofenac sodium (10mg/kg per orally) one hour before study on seventh day. The drug showed significant analgesic activity when compared to standard drug[47].

**Sombu (*Pimpinella anisum*)**

**Analgesic and anti-inflammatory activity**

Twaij et al, reported significant analgesic activity of *Pimpinella anisum* against benzoquinone induced writhing and in thermal tests[48]. Tas et al., reported that the essential oil as well as fixed oil of *Pimpinella anisum* has a significant analgesic and anti-inflammatory effects[49].

**Elam (*Elettaria cardomomum*)**

**Anti-inflammatory activity**

Hunskaar et al., Rathi N et al., Vishukanta et al., and Liu S.Y. et al., performed the formalin and Carrageenan test. The animals were divided in to 5 groups (each group consist of five animals (Rats/mice). The control group received saline solution [50,51,52,53].

**Analgesic activity**

Owoyele et al., Dharmasiri et al. and Koster et al., performed Tail immersion, Hot plate and Acetic acid induce writhing test in mice [54,55,56].

**Vaivilangam (*Embelia ribes*)**

**Anti-inflammatory activity**

Chitra M et al., studied the anti inflammatory activity of fruits of Embelia ribes as well as embelin is reported in carrageenan-induced paw edema [57].

**Annasippu (*Ilicium verum*)**

**Anti-inflammatory activity**

J.Deng et al., investigated the analgesic and anti-inflammatory actions of star anise in mice by xylene-induced auricle edema. For identification of anti-inflammatory action digestion systems of mice was separated and then xylene was injected. It was noticed that the auricle swelling of mouse by injecting xylene, decreased the threshold pain the torsion body of mice. The star anise extracts of 10 and 20 mg rough drugs·mL-1, clearly decreased the contractility of mice intestinal smooth muscles in 15 min after under the effect of acetylcholine and barium chloride. So it is concluded that aqueous extract of star anise has analgesic and anti-inflammatory effects on mice intestinal smooth muscle [58].

**Chevviyam (Root of *Piper nigrum*)**

**Anti-inflammatory and analgesic activity**

Bang JS et al., evaluated the in vitro anti-inflammatory activity on interleukin 1β stimulated fibroblast like synoviocytes obtained from rheumatoid arthritis, analgesic activity were evaluated on carrageen an induced acute paw model of pain in rats. The migration of activator protein1into the nucleus in interleukin 1β treated synoviocytes was inhibited by piperine while migration of nuclear factor κB was not a deected by piperine. The pain symptoms in rats were significantly reduced by piperine. It was concluded that piperine showed anti-inflammatory and analgesics activities in arthritis model of rat [59].

**Athimaduram (Glycyrrhiza glabra)**

**Anti-inflammatory activity**

Adel et al., studied that dehydrostilbene derivatives like $\alpha$-dihydro-3,5,4-trihydroxy-4,5-di iodopentenylstilbene have been isolated and reported as free radical scavengers. Research shows that on being broken down in the gut, glycyrrhizin exerts an anti-inflammatory action similar to hydrocorticosone and other corticosteroid hormones[60].

**Koshtam (*Costus speciosus*)**

**Anti-inflammatory and analgesic activity**

Srivastava S et al. conducted an in vivo investigation involving the oral administration of methanolic extracts from the aerial parts of *C. speciosus* at doses of 400 and 800 mg/kg. The anti-inflammatory effect of the extract was assessed using carrageenan-induced paw edema test by injection of 0.1 ml of 1% carrageenan in 0.9% saline into subplantar region of the left hind paw. Additionally, the analgesic effect was assessed using the acetic acid-induced writhing and Eddy's hot plate models. The methanol extract, when administered at doses of 400 and 800 mg/kg, exhibited noteworthy anti-inflammatory activity, with inhibition percentages of 19.36% and 40.05%, respectively, recorded at the 5-hour post-medication mark. Furthermore, writhings caused by acetic acid were reduced by 14.24 and 31.90%, respectively, and it also increased the latency period at both high and low doses that exhibited the mean reaction time at 16.60 ± 0.355 s and 14.12 ± 0.355 s, respectively, when compared with control in hot-plate test [61].

**Modi (Root of *Piper longum*)**

**Analgesic activity**

Vedhanayaki G et al., studied *P. longum* root for opioid type analgesia using rat tail-flick method and for NSAID type analgesia using acetic-acid writhing method by using pentazocine and ibuprofen as drug controls. An aqueous suspension of *P. longum* root powder was given orally to mice and rat. The study reported that *P. longum* root had weak opioid but potent NSAID type of analgesic activity [62].

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**Table 1: Herbal Ingredients of *Megasaanthi Chooranam***

| TAMIL / ENGLISH NAME [2] | BOTANICAL / FAMILY NAME[2] | PARTS USED[2] |
|---|---|---|
| Parangipattai / China root | *Smilax china* / Smilacaceae | Rhizome |
| Amukkara kizhangu / Winter cherry | *Withania somnifera* / Solanaceae | Rhizome |
| Vellarugu | *Enicostemma axillare* / Gentinaceae | Leaf, seed and bulb |
| Peraraththai / Galangal the greater | *Alpinia galanga* / Zingiberaceae | Root |
| Sangan / Mistletoeberry thorn, Four spined meneita | *Azima tetracantha* / Salvadoraceae | Leaf, root, milk and bark |
| Milakaranai / Forest pepper, Lopez-root tree, wild orange tree | *Toddalia asiatica* / Rutaceae | Leaf, bark and root |
| Kodiveli / Lead-wort | *Plumbago indica* / Plumbaginaceae | Root and bark |
| Chukku / Dried ginger | *Zingiber officinale* / Zingiberaceae | Dried rhizome |
| Thippili / Long pepper | *Piper longum* / Piperaceae | Immature berries |
| Thandri / Beleric myrobalans, Belleric myrobalan | *Terminalia bellirica* / Combretaceae | Leaf, fruit and seed |
| Kadukkai / Chebulic myrobalan, Ink nut | *Terminalia chebula* / Combretaceae | Tender fruit and fruit |
| Kanduparangi / Beetle killer | *Clerodendrum serratum* / Verbenaceae | Leaf and root |

| | | |
|---|---|---|
| Perunjchirakam / Anise seeds; Anise fruit | *Pimpinella anisum* / Apiaceae | Flower, seed and root |
| Elam / Cardamom seeds | *Elettaria cardamomum* / Zingiberaceae | Seed |
| Vaivilangam / Embelia | *Embelia ribes* / Primulaceae | Fruit, Leaf, Seed and ghee |
| Annasippu / Star anise | *Ilicium verum* / Schisandraceae | Dried fruit |
| Chevviyam / Black pepper root | *Piper nigrum* / Piperaceae | Root |
| Athimaduram / Indian or Jamaica liquorice | *Glycyrrhiza glabra* / Fabaceae | Root |
| Kottam / Costus root | *Costus speciosus* / Zingiberaceae | Root |
| Thippili ver / Long pepper root | *Piper longum* / Piperaceae | Root |

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**RESEARCH ARTICLE**

# An Expandable AI Framework with Federated Transfer Learning and Homomorphic Encryption for Real-Time Intrusion Detection – Survey

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**ABSTRACT**

Real-time intrusion detection is a necessary task to secure sensitive data and protect system integrity in the present cybersecurity landscape. However, traditional systems face significant scalability limitations and suffer from severe privacy concerns as well as detection accuracy. In this paper, we survey an artificial intelligence (AI) framework built on top of Federated Transfer Learning (FTL) and Homomorphic Encryption (HE), to improve real-time intrusion detection. FTL enables privacy-preserving model across-device training, and transfer learning allows very little data to be used for detection. It offers secure hardware execution of encrypted data computations to keep data private. Herein, a review of current research is presented and specific gaps are identified before proposing an innovative solution to enhance detection accuracy, response times and scalability while maintaining privacy.

**Keywords:** AI framework, federated learning, transfer learning, homomorphic encryption, intrusion detection, cybersecurity.

## INTRODUCTION

With the omnipresent common (cyber) threats of today, an effective Intrusion Detection Systems (IDS) is indispensable for detecting unauthorized access to your networks and identifying anomalies. Traditional IDS suffers from scalability problem [1]–[3], privacy issue and need to be updated very often as the number of threats is increasing. The advent of Artificial Intelligence (AI) has come in as a savior for improving IDS by working on enormous amounts of data, extrapolating complex patterns, and responding immediately. But they are not that good

given the limited centralized data storage and due to being dependent on large numbers of labelled training samples. In this paper, we propose a federated transfer learning (FTL) and homomorphic encryption for real-time intrusion detection using AI framework. FTL allows a privacy-preserving distributed model training across different devices without their raw data shared. Transfer learning enables the models to adapt to new environments with little data.

**Background**

It is under such a need and advantages that the extendable AI framework for intrusion detection in real-time calls for a deep dive into intrinsic concepts at play: Federated Learning, Transfer Learning, Homomorphic Encryption, and Intrusion Detection Systems. The current section tries at a detailed overview of these technologies, thus laying the ground for the proposed framework.

**Federated Learning**

Federated learning is a form of decentralized machine learning approach whereby many participating devices or organizations can train models collaboratively without sharing raw data, and it proves particularly very useful in a scenario where the privacy of data is paramount, such as in health, finance, and cybersecurity. In FL, every client trains a local model on its data and only shares model updates, such as gradients, with a central server. The latter aggregates these updates to create a global model. This preserves data privacy while contributing to the development of robust and high-performance models.

**Transfer Learning**

Transfer learning is a machine learning technique where a model that has been trained for one task can be utilized as a stepping stone to a model for a different but related activity. The potential utility of such a technique is particularly anticipated in situations where the target job lacks labeled data. This approach drastically reduces the time and computational resources required to develop models for new tasks. In intrusion detection, TL can be used to make models trained on one type of network or in an environment adaptable to another, improving the model's generalization ability. This adaptivity is very vital in cybersecurity, since new threats keep coming up every time and models should adapt quickly to the changes.

**Homomorphic Encryption**

Homomorphic encryption is a special kind of encryption that allows for computations on encrypted data and, on decryption, the results are the same as if the operations had been conducted on the plain data. This feature is very useful where data privacy is paramount and one wants to process the data securely without revealing the sensitive information. In the setting of an AI framework for intrusion detection, HE guarantees that data is kept encrypted from collection to analysis. The design solves privacy problems associated with centralizing data processing and adds an additional layer of security in distributed learning.

**Intrusion Detection Systems**

The Intrusion Detection System is one of the important segments in the modern cybersecurity infrastructures. They basically monitor against any sign of malicious behavior in network traffic and system activities. According to the detection methodology, IDSs could broadly be divided into two groups: signature-based and anomaly-based IDSs.

Signature-based IDS: Match the network traffic against a database of known attack signatures to identify known threats. This approach has been very successful at handling known threats but has proven to be weak in case of new or evolving threats. Anomaly-Based IDS: This technique detects variance that is likely to indicate the presence of new or unknown threats. Anomaly-based IDS offer greater flexibility and can detect novel attacks; however, usually, they have higher false positive rates. The bigger integration of AI, more particularly through federated learning, transfer learning, and homomorphic encryption into an intrusion detection system, is one giant step. This will provide better accuracy, adaptability, and privacy in intrusion detection systems and make them quite effective against the ever-evolving landscape of cyber threats.

**Related Works**

The integration of AI into IDS has remained a vibrant research area in the fields of machine learning, deep learning, and privacy-preserving techniques. This section shall be dedicated to the review of existing literature on Federated Learning, Transfer Learning, Homomorphic Encryption, and their applications in cybersecurity with a view to intrusion detection.

**Federated Learning in Cybersecurity**

One of the clever methods for protecting privacy in machine learning for cybersecurity has been federated learning. Its application to IDS has been the subject of numerous research studies. One of the studies, for example, demonstrated how federated learning (FL) may offer high intrusion detection accuracy while preserving privacy [7]. It has also been demonstrated that FL can be used as an IDS for the collaborative training of a model across several businesses without sharing sensitive data [6]. Another work has shown improved detection capabilities across heterogeneous environments, proposing a hierarchical model of FL to detect anomalies in IoT networks [8]. The communication burden and model convergence remain, however, major open issues [8].

**Transfer Learning for Intrusion Detection**

TL has been very much utilized in the bid to improve the performance of IDS, more particularly in cases where there is a scarcity of labelled data. Research applied TL into improving the adaptability of IDS across different network environments, which proved quite effective in reducing training time and enhancing detection accuracy in new domains [3]. Another work integrated TL with deep learning for the detection of APT and showed prominent improvement in detecting unseen attacks [5]. Most of the time, however, TL suffers because of the similarity between the source and target domains, which is a challenge not very well explored [4].

**Homomorphic Encryption in AI and IDS**

Homomorphic Encryption has been investigated as one of the prospective solutions to the privacy problem in AI-based intrusion detection. It has been shown that HE is a potential method for performing secure computations on encrypted network data, which can be used in the development of privacy-preserving IDS [2]. Another approach working on the principle of HE is applied in federated learning frameworks to guarantee that the data is encrypted during the entire training [6]. Major drawbacks towards much greater adoption of HE, even with these models, is its computational complexity and the performance overhead [2].

**Hybrid Intrusion Detection Methods**

Several studies were done that aimed to achieve the integration of TL, FL, and HE to come up with a more resilient IDS. For example, some of this work has proposed an integrated framework leveraging FL and HE for privacy-preserving and scalable intrusion detection in distributed environments [7]. Experimental results show promising results in terms of the trade-off between privacy and detection performance but point out that there is a need for more efficient algorithms to reduce computational overhead [8]. Another study focused on the application of transfer learning in federated learning for cross-domain intrusion detection, demonstrating improved adaptability across different network environments [8]. All these combined approaches are still at an infant stage, however, and a big gap exists in terms of the development of a fully integrated framework that could address all challenges of real-time intrusion detection [8].

**Proposed Framework**

First, the AI extendable framework closes the gaps in literature by putting together Federated Transfer Learning and Homomorphic Encryption to strengthen real-time intrusion detection. This paper thus details how the proposed framework shall provide a scalable and privacy-preserving solution adapted with the change of cybersecurity threats.

**System Architecture**

This framework is envisioned to have the following three main components: a module for Federated Learning, a module for Transfer Learning, and a layer for Homomorphic Encryption. All these components combine their functioning to implement a robust and secure intrusion detection system.

**Federated Learning Module:** Through this module, multiple organizations or devices train a global model of the IDS without sharing their raw data. The individual data sets train local models; just model updates are shared with a central server. Later, the central server aggregates these updates to create an improved global model that would then be distributed back to participants.

**Transfer Learning Module:** This module increases the adaptability of the IDS, allowing the fine-tuning of the global model on environments or network conditions. These are models pre-trained for other tasks from which knowledge is transferred to the target environment for adaptation, reducing the need for extensive retraining.

**Homomorphic Encryption Layer:** To obviate the chances of data exposure, this layer provides support for doing all the computations involved in the training and detection models on encrypted data itself. With that, all sensitive information is guaranteed to be perfectly safe along the line. The encryption layer is applied both on data used for training the models and data being analyzed for intrusion detection.

**Workflow and Process**

The workflow of the proposed framework can be summarized as follows:

**Information Collection and Preprocessing:** Every entity involved—one organization or one device—collects and preprocesses network traffic data. Homomorphic encryption is applied to maintain the privacy of the data.

**Local Model Training:** A local IDS model, located at each participating entity, becomes trained with the encrypted real data. Only the updates of the model get encrypted and transmitted to the central server—not the raw data itself.

**Global Model Aggregation:** The central server then aggregates the model updates of participants using encrypted federated learning techniques into a global model. This global model has the collective knowledge from all participants but still protects data privacy.

**Transfer Learning Adaptation:** Fine-tuning the global model using transfer learning techniques adapts it to specific environments or network conditions. The model will definitely work efficiently under very different situations.

**Intrusion Detection:** The final deployed model gives real-time intrusion detection in every participating environment. The model processes encrypted data to identify threats that may exist without revealing the privacy of the data.

**Benefits and Innovations**

Due to its architecture, several key advantages exist in the framework: Scalability: Federated learning ensures that the performance will not be hampered with increasing participants, irrespective of the participants. Privacy Preservation: Homomorphic encryption will ensure that data is secure throughout the process, handling privacy issues of traditional IDS. Adaptability: Transfer learning enables the framework to adapt to new environments and emerging threats very quickly and increases its effectiveness in many diverse settings. It significantly eliminates the computational overhead of extensive retraining with further pre-training and distributed learning. Collaborative Security: Federated learning enforces the collaborative contribution from parties to elevate the security posture by collective knowledge and resources. This is a new framework of complete real-time intrusion detection, which can balance the requirements of privacy, scalability, and adaptability in a changing landscape of cybersecurity threats

**Challenges and Open Issues**

While most of the advantages of the proposed expandable AI framework in real-time intrusion detection, several challenges and open issues ought to be addressed toward practical deployment and proving the effectiveness.

**Computational Complexity**

The integration of FL and HE introduces high computational complexity since both FL and HE are individually computationally expensive, and their intersection can introduce significant performance overhead. Thus, effective algorithms and hardware acceleration techniques are required to minimize the occurrence of such problems and execute them in real time.

**Overheads of Communication**

In this respect, federated learning in this case would involve high-frequency communications between different participating entities and the central server for model updates, which could easily build up into very huge communication overhead. This could, however be particularly challenging in an environment of bandwidth limited or when devices are resource-constrained. More efficient protocols for communication or compression techniques for model updates must thus be developed to reduce this overhead.

**Adaptability to Emerging Threats**

Handling of newly evolving and unknown threats. As a result of this, the framework's ability to update itself vis-à-vis newly evolving and unknown threats relies heavily on how well TL works. Unfortunately, most of the time, the success of TL relies on the closeness of the source and target domains. A very challenging continuing task is to ensure that the framework is still adaptive under the very dynamic environments where new kinds of threats keep appearing.

**Scalability in large deployments**

The higher the count of participants involved in the federated learning process, the more challenging it is to maintain the scalability of the framework. It needs to support quite a few participants without degradation either in performance or security. By studying methods of distributed aggregation and techniques of decentralized learning, the authors can resolve some of these problems related to scalability.

**Regulatory and Compliance Issues**

Federated learning and homomorphic encryption applied in intrusion detection could give rise to compliance and regulatory concerns since the laws and standards for data privacy are most relevant. In this respect, the framework would have the capability to make sure that all compliance with the necessary regulations—like the GDPR—is affected at scale across multiple regions or industries.

**Integration with Current Systems**

It may prove very challenging to integrate the proposed framework into the existing infrastructures on matters concerning cybersecurity. Because in practical implementation, the frameworks need to be compatible with and have seamless operations with the existing systems and protocols. This would require standardized interfaces and protocols for the integration process.

**User Training and Awareness**

Furthermore, user training and awareness will equally guarantee successful deployment of the framework. Users must be informed about the system's benefits and its limitations, as well as how to operate it in the best possible manner. This may involve the preparation of extensive training programs and user manuals.

Most of these challenges and open issues require careful attention to the successful implementation and effectiveness of the proposed AI framework for real-time intrusion detection.

## CONCLUSION

This paper will introduce an expandable AI framework for real-time intrusion detection based on Federated Transfer Learning and Homomorphic Encryption. The models in this framework learn attacks in a new environment and the feature space of the new environment. This is because transfer learning and federated learning make models better adaptable to new environments and threats by allowing privacy-preserving, distributed model training across multiple entities. Homomorphic Encryption protocols operate on encrypted data, and the arithmetic operation on encrypted data thus holds the protocols over an encrypted domain in the clear.

It will be based on the background surveys of related works, which would detail the strength and weakness of existing approaches and indicate the requirements for an all-around solution that respects privacy and security while being performance-focused. The presented framework can be considered a mammoth step toward the seamless, scalable, and adaptable solution of real-time intrusion detection in cybersecurity. However, these bring along computational complexity, communication overhead, and inherent security vulnerabilities placed upon federated learning and provided with homomorphic encryption. Overcoming these challenges is of paramount importance for the practical deployment and success of the proposed framework. Focus will be laid on performance optimization of the framework, methods for decentralized learning, and enhancement of resiliency in the face of adversarial attacks. We hope to make these new systems contribute to the development of resilient and privacy-preserving intrusion detection systems, which can be used effectively against the ever-changing threat environment.

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**Table 1. Benefits of Federated Learning (FL), Transfer Learning (TL), And Homomorphic Encryption (HE)**

| Feature | Federated Learning (FL) | Transfer Learning (TL) | Homomorphic Encryption (HE) |
|---|---|---|---|
| Privacy | Ensures data privacy by not sharing raw data | Data is used to adapt models without sharing it | Enables secure computation on encrypted data |
| Scalability | Scales with the number of participating devices | Reduces training time for large datasets | Requires efficient computation for large datasets |
| Adaptability | Enhances generalization across diverse environments | Allows models to adapt to new tasks/environments | Preserves privacy in adaptable models |
| Computational Overhead | Moderate, dependent on number of participants | Low, but depends on similarity of domains | High, due to encryption/decryption processes |

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**RESEARCH ARTICLE**

# Extraction of Alginate from Brown Seaweed *Sargassum natans* and Its Potential Application as an Ecofriendly Biopolymer and Bioadsorbant

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## ABSTRACT

Marine macro algae *Sargassum natans* is common in Indian coastal regions. *S. natans* was purchased and was processed for the extraction of alginate. Alginate is linear polysaccharide present in the cell wall of brown seaweed. The production of alginate bioplastic will help in reducing the conventional plastics and their toxic effects on human life. The seaweed used in the study *Sargassum natans* was subjected to various pretreatment with formaldehyde, hydrochloric acid and sodium bicarbonate before extraction of alginate. This extracted alginate was used as a starting material for the production of bioplastic film. Synthesis of biodegradable films was done using alginate compiled with glycerol, PVA and vinegar. Study of degradation was conducted by burying it under a fertile composite soil and observed for the changes at regular intervals. Because of high microbial load and fertility, compost soil was chosen. The soil burial test was carried out for a period of 30 days. Changes like loss of color, texture and weight were observed upon degradation.The water absorption tendency of alginate bioplastic film was tested and it showed notable percentage of water absorption. This biodegradable plastic can easily be disposed into environment without any kind of toxicity. Further the extracted alginates were converted into calcium alginate beads by treating it with aqueous solution of calcium chloride. Heavy metal solution was prepared and it was treated with extracted alginate beads. The alginate beads were tested for its characteristic adsorption of divalent ions. Adsorption of heavy metals was confirmed by FTIR. This proves that the alginate extracted from marine macro algae *S.natans* can be used as starting material for the synthesis of eco friendly bioplastic and bioadsorbant of heavy metals.

**Keywords:** *Sargassum natans*, alginate, bioplastic, biodegradable films, bioadsorbant

## INTRODUCTION

Polymer plays a vital role in human life but they are difficult to degrade by natural methods. The incineration of the polymers also releases poisonous gases in the atmosphere and they cause toxic effect to humans and environment. Bioplastics derived from renewable biological sources like microorganisms, plants and seaweed are alternate for synthetic polymers. Bioplastics are non-toxic, easier to recycle requires less energy to manufacture and independent of fossil fuels. Bioplastics are degraded to carbon di oxide and water in aerobic conditions, methane and carbon di oxide are produced under anaerobic conditions. Algae have the potential for production of biopolymers. Seaweed polymer could be the solution for the reduction of plastic waste and environmental hazards. Brown seaweed gains importance because they possess a large amount of alginate, which has a high chemical affinity towards divalent metals. Alginate is abundant in brown seaweed *Sargassum sp*. The alginate extracted from brown sea weed biomass are used as potential bioadsorbant for heavy metals (Trica *et al.,* 2019). On reacting with divalent ions like iron, manganese, magnesium, and aluminum, and calcium, alginate forms gel. Alginate can be stable from 0-100°C.

Seaweed polymer attracted interest of most scientists as a film-forming substance. Due to its hydrocolloidal characteristics it is manufactured into an environmentally friendly film. *S.natans* is bushy seaweed with narrow leaf blades which are golden brown with toothed edges. Due to its advantages and availability of *S.natans,* the present study was attempted to extract and characterize alginate from the marine macro algae and investigate its potential application as heavy metal absorbent and as a potential substrate for bioplastic film.

## Materials and Methods

**Collection of Sample**

The dried mass of marine macro algae *Sargassum natans* (Fig.1) was purchased from Gujarat.

**Pretreatment of seaweed:**

The dried biomass of seaweed was weighed up to 20g (2 sets) and washed repeatedly with tap water to get rid of large sand particles and dust molecules. It was then soaked in distilled water for one hour. Later it was drained and chopped into small pieces. Following this seaweed was soaked in 300ml of 1% calcium chloride solution overnight at room temperature for 18 hrs. The treated seaweed was again washed with distilled water and soaked in 4% formaldehyde for 2 hrs. Treatment with formaldehyde helps in discoloration of the seaweed.

**Acid Treatment:**

The formaldehyde was drained and the seaweed was washed with distilled water. The treated seaweed was transferred to a beaker containing 5% hydrochloric acid (HCl) for 1 hour and washed again with distilled water. Treatment with mild acid helps to remove other unnecessary compounds like phenolic compounds and also epiphytes. Acid treatment also helps in demineralization and delipidisation of the seaweed

**Extraction of Alginate**

The extraction of alginate was followed by a standard procedure described by Haug *et al*., (1964) and Chee *et al*., (2011) with slight modification. The acid treated seaweed was washed and 300ml of 3% $Na_2CO_3$ (Sodium bicarbonate) was added to the beaker and left undisturbed for 1 hour. Alginate dissolves and precipitate in the form of sodium salts when treated with sodium bicarbonate. This mixture was stirred well for another 1 hour and 250ml of distilled water was added to the sodium bicarbonate and seaweed solution this was then left for overnight at room temperature. The solution was centrifuged at 3500 rpm for 3-4 minutes. The sediments are discarded and the supernatant was collected in a separate container. Container containing supernatant with 90% ethanol. The alginate forms gel when treated with ethanol. The gel was then filtered using a mesh cloth and washed repeatedly with ethanol and dried (Fig 2 & 3)

**Preparation of Alginate beads**

**Preparation of calcium chloride soution:**

To prepare alginate beads aqueous solution of calcium chloride ($CaCl_2$) was prepared. To make 4% calcium chloride solution 4 grams of $CaCl_2$ was weighed and dissolved in 100 ml of distilled water and stored in air tight container

**Immobilization of alginate**

5g of extracted alginate was dissolved thoroughly in 100 ml of distilled water. This alginate was then dripped into the aqueous solution of calcium chloride using a syringe needle (Fig. 4a). The prepared alginate beads were kept in contact with calcium chloride solution for 24 hours. Then the beads were filtered and washed with distilled water and air dried (Fig.4b).

**Preparation of Heavy Metal Aqueous Solution**

1g of $FeSo_4$ (Iron sulphate heptahydrate), $CuSo_4$ (copper sulphate pentahydrate), $ZnSo_4$(zinc sulphate heptahydrate), $MnCl_2$(manganese chloride tetra hydrate). $CoCl_2$
(Cobalt chloride hexahydrate) was weighed and dissolved in 500ml of deionized water.

**Preparation of Film**

The alginate polymer film was prepared by dissolving the extracted alginate in distilled water. Glycerol / Poly vinyl alcohol (PVA) or both are added to the beaker containing extracted alginate along with this non-fruit vinegar was added with little amount of mild HCl and stirred for 15 minutes. After stirring the mixture was maintained at 160˚C for 10-15 minutes. It formed a gelatin like solution. The solution was poured in an aluminum foil sheet. Allowed to cool for 24hrs. After cooling the film was kept at hot air oven at 60˚C for 2-3 hours to get a dry film (fig 5 a, fig 5 b).

**Characterization of Alginate**

**FTIR Analysis of Alginate Beads**

The FTIR was mainly used to identify the functional group of the sample. FTIR was used to detect the surface functional group of alginate beads. It was done before and after the treatment of heavy metals. The samples were named properly as sample 1(before treatment) and sample 2 (after treatment). FTIR was performed as per the standard procedure.

**Biodegradation of Alginate Film**

To examine the degradability of alginate bioplastic film Soil burial test were conducted. Degradation is promoted by number of microorganisms and their enzyme produced by them. This can occur at aerobic and anaerobic condition. Degradability may lead to the removal of waste either fully or partially from the environment. Compost soil was collected in a container. The soil moisture, humidity and pH were monitored Two cardboard of size 10×10cm were taken one was pinned with synthetic polythene sheet (control) and another with alginate sheet (test) (Fig. 6). Both the test and the control were buried in the compost soil. In the interval of 10 days film was taken away from soil cleaned and observed for changes. Changes in the color, weight, texture and measurements were noted. Soil burial test lasted for 35 days.

**Analysis of Water Absorption Capacity**

The test is carried according to ASTM D570 METHOD (standard test method for water absorption of plastics). The film are cut into pieces of size 20mm×20mm kept in hot air oven at 60˚c for 2 hours. Weight of the film is measured ($W_{dry}$) before it is dipped in water. Dried films were dipped in distilled water at room temperature for 24 hours. After that it was removed and wiped gently by blotting paper and weighted ($W_{wet}$) to determine the water absorbed by the films. The bio absorption quality ($W_a$) was determined by the formula,

$$\%W = \frac{wet - dry}{dry} \times 100$$

## RESULTS

**FTIR Analysis of Alginate Beads:**
**A**nalysis of alginate beads for its adsorption of heavy metals was carried out by FTIR.

**Sample1 (before treatment)**
In prepared alginate polymers beads, finger print region was observed with prominent peak variations. Peak at 1400cm$^{-1}$ band denoted strong S=O stretching sulfate 1248 cm$^{-1}$ denoted the C=N stretch of amine, peak at 1048cm$^{-1}$ denoted the presence of aliphatic ether peak at 650cm$^{-1}$ attributed the C-Br stretching compound. Peak at 2145cm$^{-1}$ denoted the presence of N=C=N stretch carbodiimide compound also a prominent broad peak at 3350cm$^{-1}$ attributed the presence of OH group. (Fig. 7 a)

**Sample 2 (After treatment)**
FTIR- Spectra of treated alginate polymer beads were also observed with variations in finger print region when compared with untreated alginate polymer peak at 2000 cm$^{-1}$ confirms the presence of C=C=N stretching of ketenimine. Peak at 1600cm$^{-1}$ and 1500 cm$^{-1}$ represented strong N-O stretching nitro compound. Also peak at 1350 cm$^{-1}$ denoted alcohol group and 1215 cm$^{-1}$ peak denoted amine group presence. Peak at 685 cm$^{-1}$ corresponded at C-Br stretch at a halogen compound. (Fig. 7 b)

**Soil Burial Test (Biodegradation Test)**
Soil burial test was carried out to test the biodegradability of alginate bioplastic film. Soil microorganisms are responsible for the biodegradation of alginate bioplastic film. Degradability of the polymer is an ideal characteristic of a bioplastic for its application purpose. The changes were noted at the interval of 10 days (Fig. 8-11; Table 1)

**Water Absorption Test;**
Water absorption is the major characteristic of a biopolymer for is degradability and its application in various industries. The hydrophilicity of alginate bioplastic film was calculated by measuring the absorption capacity of the film. ASTM D570 METHOD (standard test method for water absorption of plastics) was followed in this study.

Alginate sheet 1: dry weight-10.80 g wet weight: 52.51g
Alginate sheet 2: dry weight-11.66g wet weight: 56.04g

## DISCUSSION

The large production and application of conventional plastic has led to hazards and environmental pollution hence bioplastic is introduced as an alternative because of its biodegradable properties. Petro chemicals-based plastics which are resistant to biodegradation and which are environmentally damaging can be replaced by microbial biopolymers. Alginate is a phyco colloid extracted from brown seaweeds used as a starting material for bioplastic film. Algae have the advantage of using smaller amount of nutrients during the process of production of biopolymers. Sargassum is a type of brown seaweed that produces high amount of alginate which is currently in high demand. There are a number of studies employing the application of alginate in producing bioplastic. Seaweed has been reported to be effective heavy metal sorbents and has been proposed as low cost biosorbants to be used in industrial application.

The brown seaweed *S.natans* was chosen in this study as a source of starting material for bioplastic and for heavy metal sorption because of the high availability in Gujarat coast as suggested by Rajendran *et al*.,2012. After pretreatment process the obtained average yield of sodium alginate recorded in this study was 25%. This is considered as the acceptable range of industrial standard of alginate yield of 13 to 38%. Bertagnolli *et al*.,2014. Lim et

al., 2018 have reported yields in the similar range in their studies where *Sargassum siliquosum* was used as a starting material for the synthesis of bioplastic. Martins *et al*., 2017 have reported *Nostoc ellisosporum* and Spirulina as a potential source of crude biopolymers. Hence to the best of our knowledge this is the first study employing *S. natans* as a highly efficient source of biopolymer.

The percentage of matter loss during extraction was due to different treatment with formaldehyde, Hydrochloric acid and sodium bicarbonate. Alginate was extracted in its sodium form using sodium bicarbonate and with formaldehyde treatment prior to extraction. Phenolic compounds and plants matter polymerized upon addition of formaldehyde. This lowers the solubility and minimizes the release of phenol content into the final alginate sample.
The present study employed the usage of Glycerol and PVC as the plasticizers to obtain high strength elasticity smooth surfaced polymeric film. This is in conformity with Kipngetich *et al*., 2013. However, Hillary *et al*., 2013 in their study employed propane 1- 2-3 triol as a plasticizer since they found biopolymers with plasticizers were brittle and rigid. The bioplastic film did not degrade under room temperature indicating their significance for usage in normal storage conditions.

The synthesized alginate biofilm was subjected to water absorption and biodegradability test. Water absorption is an important for the biodegradable materials. The water absorption capacity of bioplastic film was evaluated by measurement of film surface. A maximum water absorption of film was observed in current study in 24 hr period.
Biodegradation of the film by soil burial test under compost soil for a 30 days study period indicated degradation of the film resulting in the complete degradation of the film to about 65% of its weight and thickness. While the synthetic polymer shows no observable change in 30 days study period. The study hence strongly suggested the use of alginate polymer as an environmentally friendly polymer which usage to be encourage and adopted. Among various macro algae brown algae contain alginate which have chemical affinity towards divalent ions hence the present study tested the alginate beads for their bioadsorption capacity. The alginate extracted from *S. natans* as was jellified in the form of calcium alginate to be used as bioadsorbant. Metal ions are removed by ionic exchange. FTIR analysis was carried out to study the changes after bioadsorption of metallic ions by the alginate. The following observations were made in the samples before and after treatment.

For sample 1 & 2 the FTIR analysis was shown to be Fig. shows Peak at 2145cm-1 N=C=N stretch carbodiimide compound was known as a membrane active agent responsible for the loss of ion homeostasis on the cell membrane causing the inhibition of photorespiration. Most of the imide derivatives were interacted with cell membrane and release of intracellular constituents Ohya *et al*.,1997 further C=C=N stretch of ketamine at peak 2000 cm-1 confirmed the ion exchange property. The presence of imide group confirms the absorption capacity of alginate functional group. Conversion of carbodiimide to ketoimide.

When a single halogen is present it usually exhibits free rotation, which would normally mean that there are no performed spatial orientations for the molecules. Large number of granular microspheres with a uniform size were dispersed on the surface of the alginate. Probably this may be due to the presence of carboxyl group of alginate (Coo-) which can interact with amino groups (NH3 +). Usually, band is assigned to the stretching vibrations, asymmetric stretch in the case of cumulative double bonded compound. Whereas symmetric stretch is typically weak and is not diagnosed at the group. The FTIR analysis shows that the main chemical group involved in biosorption for *S. natans* were carboxylic alcoholic and amino group which is in conformity earlier study Kleinubing *et al*., 2011 who studied metals sorption by calcium alginate beads from *Sargassum filipendula*. This current study focused on the synthesis of biopolymers from *S. natans* and to test the bioadsorption capacity of extracted alginate. With the result obtained it can be concluded that *S. natans* as a potential candidate capable of yielding alginate in high concentration which can be used as an alternate source to petrochemical plastics. Bioplastics from seaweed are in the early stage of development but definitely holds significant promise in developing biodegradable, eco -friendly and sustainable plastic for the future.

By analyzing the FTIR result it can be concluded that the presence of imide group confirms the absorption capacity of alginate functional group. The functional group extracted from the algae may play an important role in bioabsorption of metallic ions.

## CONCLUSION

The result from the current study designates that the extraction of alginate from the brown seaweed *Sargassum natans* was significantly high up to 25%. Compared to the synthetic plastics the synthesized alginate bioplastic film showed noteworthy biodegradability up to 65%. The soil burial test proved the degradability of film which was the main idea of this study. It also displayed remarkable water absorption capacity and hydrophobic characteristic of alginate bioplastic film. The extracted alginate beads upon treatment of calcium chloride showed superabsorbent of heavy metals. FTIR analysis describes the presence of functional group of the alginate beads before and after treatment with divalent ions. Production of bioplastic from *S. natans* has been successfully performed. The research presents *S. natans* as a cost-effective remarkable source for sustainable bioplastic in the future and also as a potential bioadsorbant for removal of heavy metals. To the best of our knowledge the present study could be considered to be the first research work in the synthesis of bioplastic from *S. natans.*

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**Table 1: Changes observed in the alginate bioplastic**

| No. of Days | Texture | Color | Weight | Degradation of Sheet in Cm |
|---|---|---|---|---|
| At the day of burial | Rigid strong and thick | Pale yellow to light brown | 10.86g | No degradation |
| 10 days of burial | Change in texture, soften and jelly like | No adverse color changes | 9.21g | 3-4cm |
| 20 days of burial | Became thin weak and soften | Color changed to dark brown in color | 7.24g | 9-10cm |
| **30 days of burial** | Shrunken and film became more thin | Dark brown in color | 7.2g | Most part were degraded when compare to synthetic plastic (17-20cm) |

**Fig. 1: Dry mass of *S.natans***

**Fig 2: Extracted alginate**

**Fig 3: Precipitation and filtration of alginate by the treatment of ethanol**

**Fig 4 (a): Alginate beads preparation with calcium chloride**

**Fig 4 (b): Alginate beads**

**Fig 5 (a): Alginate sheet with Glycerin and Polyvinyl alcohol**

**Fig 5 (b): Alginate sheet with glycerin**

**Fig 6: Synthetic polythene sheet vs. alginate bioplastic**

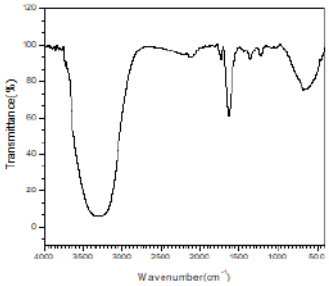


**Fig 7 a: FTIR graph of alginate beads before treatment with heavy metals**

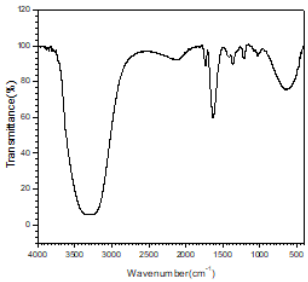


**Fig 7 b: FTIR graph of alginate beads after treatment with heavy metals**

**Fig 8: Day 1 of soil Burial**

**Fig 9: After 10 days of soil burial**

**Fig 10: After 20 days of soil burial**

**Fig 11: After 30 days of soil burial**

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**RESEARCH ARTICLE**

# A New Approach for Scalable Synchronization and Efficient Routing in Industrial IoT for WSNS: A Survey

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## ABSTRACT

Wireless sensor networks (WSNs) are becoming more important for data collection and transmission in industrial environments as IIoT applications continue to grow rapidly. But, new ways of thinking about scalable synchronization and efficient routing are required because of the problems that WSNs already have, such low bandwidth, energy limits, and changing network topologies. In order to solve synchronization and routing challenges in WSNs for IoT applications, this review study seeks to provide a thorough overview of current developments and new solutions. We begin with a thorough examination of the role of synchronization in guaranteeing accurate data, time synchronization techniques, and their practical use in businesses. We take a look at both older and more modern approaches of synchronization, comparing and contrasting Reference Broadcast Synchronization (RBS) with NTP and PTP, and talking about the pros and cons of each. In addition, this overview emphasizes how fog computing and edge computing may improve routing efficiency and synchronization accuracy by decreasing data transmission volumes and latency. We highlight the advantages of fog-assisted routing algorithms and edge-based synchronization methods for processing data in real-time and making decisions at the network's periphery.

**Keywords:** Efficiency routing, Scalable, Time synchronization protocols, Wireless Sensor Networks

# INTRODUCTION

In recent years, WSNs have garnered attention from several fields, including smart cities, healthcare, industrial automation, and environmental monitoring [1-2]. The tiny, resource-limited sensor nodes that make up these networks work together to gather, analyze, and send data for a variety of reasons [3-4]. It is not easy to create trustworthy algorithms for WSNs due to their specific properties, such as their limited energy resources, changeable network architecture, and communication limitations [5-6]. Our research will center on developing new algorithms and approaches for WSNs and resolving critical issues with them so that they can scale better, perform better, and be more efficient. There are three parts to the study, and each one delves into a different facet of WSNs [7-8]. The first step is to achieve time-based synchronization in WSNs by using the Reference Broadcast Synchronization (RBSync) method in conjunction with the Flooding Time Synchronization Protocol (FTSP) model [9–10]. For coordinated data collecting, event detection, and time-sensitive applications, it is essential that sensor nodes be in sync with one another in terms of time. In order to facilitate precise coordination and data interchange, we will examine how well the FTSP-RBSync algorithm works in setting up a common timebase across sensor nodes [11, 12].

Using geographic routing concepts in conjunction with the creation of a Minimum Spanning Tree (IMST), the GPSR-IMST routing algorithm is proposed in the second phase. The IMST structure optimizes routing pathways to decrease energy consumption and boost efficiency [13–14], while geographic routing takes use of the sensor nodes' geographical information. An effective and trustworthy routing technique for WSN data transmission based on geographic information and the IMST structure is the goal of this study [15]. The third stage is focused about solving the scalability problems in WSNs by creating the PVH-DHT algorithm. CAN's multi-dimensional Cartesian coordinate system and Pastry's prefix-based routing strategy are combined in PVH-DHT to provide fast search and storing operations [16–17]. As a reliable and extensible solution for data management in WSNs, PVH-DHT will be tested for its scalability, fault tolerance, and efficient use of resources [18-21]. Extensive simulations and evaluations will be conducted to thoroughly assess the performance, effectiveness, and practical feasibility of the proposed algorithms [22-25]. The research outcomes aim to make significant contributions to the advancement of WSNs by tackling critical challenges and offering innovative solutions for time synchronization, efficient routing, and scalable data management [26-29]. These results have the potential to make WSNs simpler to install and utilize while also increasing their efficiency, utility, and dependability across a variety of industries [30–31].

**Background study**

**Survey on time based synchronization**

Chemodanov, D. et al. (2019) Improving geographical routing methods for incident-supporting applications in edge computing that are based on the Internet of Things was the main emphasis of these authors' work. Improving scalability, reliability, and stability, the objective was to provide continuous high-speed data transmission to an edge cloud gateway. Dian, F. et al. (2017) Achieving temporal synchronization between BLE devices is the main objective of this study. To do this, the time difference between events produced by BLE master and BLE slave during connection creation is analyzed. After painstakingly measuring the time difference using an oscilloscope, the author devised a circuit to facilitate the measurement process. Ghaderi, M. et al. (2019) the author have developed a fuzzy-based geographic routing protocol called FGAF-CDG. It utilizes a hexagonal virtual grid architecture, inspired by the GAF protocol. In addition, the author implemented the CDG method to enhance energy efficiency and optimize load distribution in the network, utilizing the compressive sensing technique.

Godor, I. et al. (2020) these authors research provides a comprehensive explanation of how 5G enables seamless time synchronization in industrial automation, even when integrated with wireline networks. The paper primarily examines the integration of 5G and TSN in future wireline industrial communication networks. It delves into various factors that must be taken into account to ensure precise time synchronization between the two domains. Hadi, N. T., & Wibisono. (2019) Wireless sensor networks play a crucial role in the Internet of Things (IoT), finding applications in diverse fields like healthcare, military operations, and traffic management. The WSN network was made up of a sink

node and a group of sensor nodes that were spread out in space. Given the constraints of sensor nodes, it was crucial to implement efficient routing protocols in order to maximize the network's lifespan.

**Survey on Geographic Routing**

Hasan, K. et al. (2018) There was a paucity of knowledge on the accuracy requirements of GNSS-based time synchronization in VANET. In this article, we have looked at why GNSS time synchronization is important in VANET and what circumstances are required for it to work. An extensive and painstaking investigation has uncovered many VANET uses that depend substantially on time synchronization. The need or desirability of time synchronization was determined for various uses. Hasan, M. et al. (2018) So that the Internet of Things (IoT) may function normally with a uniform distribution of sensor nodes, the author must do away with the single-hop routing scheme. Whether it's better to route via a smaller number of longer hops or over numerous short hops was a key topic that was presented. In order to surpass nearest-neighbor routing models, multi-hop geographic routing green systems suggest transmitting as far as feasible.

**Survey on efficient data transmission in wireless sensor networks**

Liu, C. et al. (2018) since the transmission direction was not taken into account by the standard GOR, it may not be able to manage some unique situations in the widely used strip network structures. So, for the strip network topologies, the author created a new GOR mechanism the author dubbed EasyGo. Mahmood, A. et al. (2018) The third axis of the URLLC-model might be ultra-tight synchronization, which is important for time-critical applications. Smart grids and industrial automation, two main uses of URLLC, need precise device synchronization with a reference clock. Due to the fact that 5G URLLC would not replace current industrial bus systems, new connections to both wired and wireless networks were necessary. Naghibi, M., & Barati, H. (2020) Using mobile sinks was one way to address the issue of data transfer in WSNs from sensor nodes to the sink. In this work, the author provide a routing algorithm that makes use of mobile sinks. Two movable sinks, together with the right mobility pattern, have really been used. Phan, L.-A., & Kim, T. (2021) To solve the issue of average-based consensus time synchronization techniques' delayed convergence, VTSP, a new protocol, was suggested in this article. Virtual topologies with better algebraic connectedness than physical topologies were used to conduct the consensus process in VTSP. Connecting nodes in close proximity to one another via virtual linkages creates the virtual topology. Sangaiah, A. et al. (2021) the first node in a WSN will be the one to transmit the route demand. Data was sent along a route that the network discovered using the appropriate protocol, which began at the source and ended at the destination. But this paper uses a different routing search approach. In order to facilitate network operations, this article primarily contributes to the topic of creating a predetermined routing tree.

Şen, S. et al. (2021) more robust possibilities for eHealth applications were made available by the IoT. Enhancing the efficacy of eHealth systems was possible via interdisciplinary research. This work has conducted experiments on intra- and inter-WBAN designs for use in pandemic scenarios, using AODV, geographic routing algorithms, and IEEE 802.15.6 based on CSMA-CA. Tian, Y.-P. et al. (2021) the author look at how time synchronization techniques fare when faced with unpredictable, limited communication delays. To start, the author prove that CBTS algorithms' offset estimates were not converging in networks with two or more root nodes, regardless of how fast the drift estimates converge, even if the convergence rate was O(1/k). After that, in order to ensure that the offset estimate remains confined under conditions of random bounded delays, the author provide the DCBTS method. Wang, H. et al. (2018) The three-step time synchronization mechanism proposed in this letter is based on the maximum consensus technique for clustered WSNs and has little overhead. The suggested approach has been shown successful via simulation results. Zhu, H. et al. (2020) many industrial automation and vehicle applications rely on TSN to satisfy their low latency and real-time data transfer needs. The efficiency and effectiveness of the TSN and the network were affected by how precisely and consistently the clocks were synchronized.

**EXISTING METHODS**

**Popular methods**

**Flooding Time Synchronization Protocol**

Based on the idea of Reference Broadcast Synchronization (RBS), in which a specific node broadcasts synchronization beacons at regular intervals to serve as the network's time reference, the Flooding Time Synchronization Protocol (FTSP) is a method for synchronizing times in Wireless Sensor Networks (WSNs). In response to these beacons, nodes use clock filtering algorithms to minimize the impacts of drift and outliers and bring their clocks into synchronization with the reference node. Accurate time coordination is essential for WSN applications like data fusion and event coordination, and FTSP assures synchronization stability by adjusting to changing network topologies. It also keeps energy efficiency high by eliminating superfluous synchronization actions.

**Reference Broadcast Synchronization**

Distributed systems, and Wireless Sensor Networks (WSNs) in particular, employ Reference Broadcast Synchronization (RBS) to ensure that all of its nodes are maintaining precise and synchronized time. An RBS synchronization beacon, with the reference broadcast coordinator's (RBC) current timestamp and sequence number, is transmitted at regular intervals by a specified node. In order to get their clocks in sync with the RBC's, other nodes in the network keep track of when these beacons are received, as well as the sequence number. Long-term synchronization stability is ensured by RBS via the use of statistical methods such as clock filtering and outlier elimination, which decrease clock drift and synchronization faults. This method is crucial for WSN deployment applications that need accurate time coordination and data correlation across dispersed nodes.

**Greedy Perimeter Stateless Routing**

In order to create communication pathways between nodes in wireless ad hoc networks and WSNs, the routing protocol known as GPSR is often used. This approach does not depend on pre-established routing tables or global network information. Each GPSR node uses its own unique set of local data to determine its own unique route. This decentralized and localized approach is what makes GPSR so effective. Two modes of operation are available to the protocol: perimeter forwarding and greedy forwarding. With greedy forwarding, a node will try to reduce the number of hops needed to reach its target by sending packets to its neighbor that is geographically nearest to it. The protocol transitions to perimeter forwarding whenever greedy forwarding hits barriers or local maxima; in this mode, the packet travels around the network's periphery until it finds a viable route to its destination. GPSR has several benefits, including scalability, reduced routing overhead, and flexibility to changing network topologies. Nevertheless, it could encounter problems in situations involving empty spaces or network segments, necessitating supplementary fault tolerance and resilience measures.

**Minimum Spanning Tree**

An essential idea in graph theory and network architecture, a Minimum Spanning Tree (MST) minimizes the overall cost or weight of edges in linked networks, allowing for efficient communication channels. In computer networks, MSTs help in routing, broadcasting, and allocating resources by ensuring that all nodes can be reached via a cycle-free tree structure. Built using algorithms like Prim's and Kruskal's, MSTs are essential for designing and managing efficient, scalable, and fault-tolerant network architectures. They are used in hierarchical routing, load balancing, and network protocols like spanning tree protocols.

**Prefix-VHash**

For distributed systems and databases, Prefix-VHash is a data structure and hashing algorithm that efficiently stores and retrieves hierarchical key-value pairs. By using hierarchical key organization with variable-length prefixes, it maximizes storage consumption, decreases redundancy, and improves memory efficiency. By limiting the hashing process to key prefixes only, Prefix-VHash reduces the likelihood of collisions and allows for quick, predictable key lookups. This method shines in contexts like file systems or classification schemes where keys display hierarchical structures or share common prefixes. Prefix-VHash is an excellent choice for organizing and accessing hierarchical

data in distributed contexts because to its many benefits, including optimal storage, rapid retrieval times, organized data in a hierarchical fashion, and reduction of collisions.

## DISCUSSION

Despite its centrality to IIoT data collection and transmission, Wireless Sensor Networks (WSNs) confront obstacles such bandwidth constraints and changing topologies, calling for creative solutions to synchronization and routing. With an emphasis on the importance of synchronization for data accuracy, this survey paper seeks to offer a comprehensive overview of recent advancements in these areas. It delves into protocols such as Reference Broadcast Synchronization (RBS), Network Time Protocol (NTP), and Precision Time Protocol (PTP), examining their advantages and limitations in industrial settings. In addition, the survey explores how fog computing and edge computing optimize data processing and decision-making at the network edge, which is essential for real-time IIoT applications. It showcases fog-assisted routing algorithms and edge-based synchronization solutions that improve routing efficiency and accuracy.

## CONCLUSION

Finally, despite problems like fluctuating topologies and insufficient bandwidth, WSNs are becoming more important in industrial environments due to the fast growth of IIoT applications. In this review, we have looked at the current state of the art and future directions of IIoT routing and synchronization for WSNs, with a particular emphasis on protocols such as RBS, NTP, and PTP. We have also discussed how edge and fog computing can improve the accuracy and efficiency of routing and synchronization. The successful integration and dependable functioning of IIoT systems in industrial settings may be achieved by WSNs by using these advancements, which lead to better data accuracy, lower latency, and optimum resource use. The future of industrial automation and smart manufacturing will be greatly influenced by WSNs, and their progress will be driven by ongoing research and development in these fields.

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**Table 1: Comparison table for existing work done by authors**

| S/NO | Author | Years | Methodology | Advantage | Limitation |
|---|---|---|---|---|---|
| 1 | Alomari, A. et al. | 2017 | Wireless sensor networks | The model improves localization accuracy by providing localization information to all nodes. The mobile anchor coordinates node information exchange by strategically traveling a static path. | Choosing the optimal static path for the moveable anchor may be tough. Finding a strategy that maximizes localization accuracy while minimizing energy consumption and considering precision and localization ratio metrics may need processing resources. |
| 2 | Chelouah, L. et al. | 2017 | Mobile wireless sensor networks | There was a paucity of knowledge about the precision requirements for GNSS-based time synchronization in VANET, and the study categorizes localization options according to methodology, anchor-based or cooperative strategies, node mobility state, and information state. In this article, we have looked at why GNSS time synchronization is important in VANET and what circumstances are required for it to work. An extensive and painstaking investigation has uncovered many VANET uses that depend substantially on time synchronization. The need or desirability of time synchronization was | Due to the area's growth, the research may not include all MWSN localization methods. The analysis may not incorporate new algorithms and techniques developed after the report was published. |

| | | | | | |
|---|---|---|---|---|---|
| | | | | determined for various uses.. This category helps academics and practitioners understand the different MWSN localization algorithms and pick the optimal one for their purposes. | |
| 3 | Erdemir, E., & Tuncer, T. E. | 2018 | alternating minimization | One benefit of the recommended route planning algorithms for mobile anchor (MA)-aided localization in wireless sensor networks was that they can localize sensors with fewer anchors and shorter paths. | The offered algorithms' efficacy depends on known anchor placement. Localization algorithms may function poorly if anchor placement was inaccurate. |
| 4 | Han, G. et al. | 2017 | Scan and Localization algorithm with a Mobile Anchor node based on Trilateration | The SLMAT approach employs trilateration and a moveable anchor node to estimate the positions of unknown network nodes. | The SLMAT algorithm considers the moveable anchor node's energy usage during mobility. |
| 5 | Kouroshnezhad, S. et al. | 2018 | Optimal Priority based Trajectory with Energy Constraint | OPTEC optimizes movable anchor trajectory planning using MILP. Localisation precision, energy limits, and location uncertainty were utilized to discover the trajectory that maximises localisation performance while considering the mobile anchor node's limited resources. | MILP optimization may increase computing complexity in the OPTEC approach. Solving MILP problems may be computationally difficult for large sensor networks. |

**Table 2: comparison table for existing methods**

| Algorithm | Merits | Demerits |
|---|---|---|
| Flooding Time Synchronization Protocol | FTSP operates in a decentralized manner, allowing nodes to synchronize their clocks without the need for a central time server. This reduces the dependency on a single point of failure and improves robustness in WSN deployments. | FTSP relies on periodic time synchronization messages (beacons) sent by nodes, which can contribute to increased network traffic, especially in large-scale deployments. This continuous communication may lead to higher energy consumption and reduced network lifetime. |
| Reference Broadcast Synchronization | RBS achieves high synchronization accuracy by leveraging the concept of reference broadcasts. In RBS, a designated node called the Reference | RBS may experience an initial synchronization delay when a node joins the network or after network reconfiguration. During this period, |

| | | |
|---|---|---|
| | Broadcast Coordinator (RBC) periodically broadcasts synchronization beacons. | the node needs to receive and process multiple synchronization beacons to adjust its clock, which can temporarily delay accurate synchronization. |
| Greedy Perimeter Stateless Routing | GPSR is designed to be efficient in terms of routing overhead and resource utilization. Using a greedy forwarding method, it directs packets to the next node with the greatest proximity to their final destination. This approach minimizes the number of hops and reduces latency in data transmission. | GPSR's greedy forwarding strategy can lead to suboptimal routing decisions in certain scenarios. Nodes may get trapped in local optima, where the closest neighbor in terms of Euclidean distance is not necessarily the optimal next hop in terms of network connectivity or path length. |
| Minimum Spanning Tree | An MST ensures that all nodes in a graph are connected with minimum possible edges. This property is essential in network design to establish communication paths between nodes while minimizing the overall cost or distance. | MSTs are static structures that do not dynamically adapt to changes in network topology or traffic patterns. In dynamic networks or scenarios with frequent topology changes, maintaining an optimal MST may require frequent recomputation, leading to overhead and potential disruptions. |
| Prefix-VHash | Prefix-VHash optimizes storage efficiency by grouping keys with shared prefixes together. Similar keys are likely to have common prefixes, leading to compact storage and reduced redundancy in distributed storage systems. | Like any hash-based data structure, Prefix-VHash may encounter collisions where different keys hash to the same prefix. Collision resolution strategies such as chaining or open addressing may be required to handle collisions and maintain data integrity. |

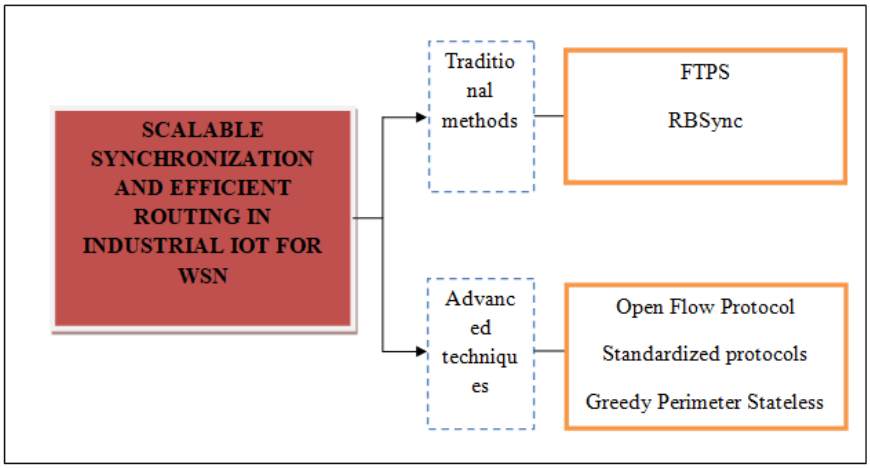


Figure 1: Methods for scalable synchronization and efficient routing in industrial IoT for WSN

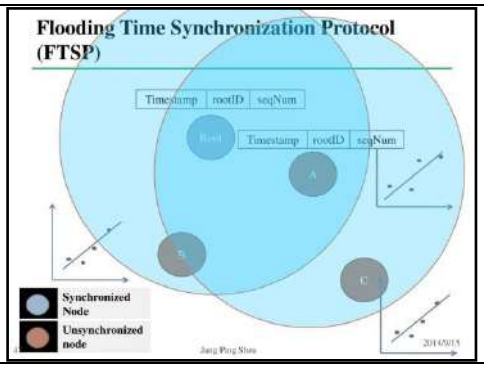


**Figure 2: Flooding Time Synchronization Protocol [Source: https://www.slideserve.com/jayden/chapter-6-time-synchronization]**

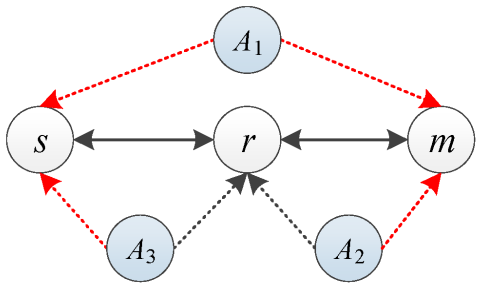


**Figure 3: Reference Broadcast Synchronization [Source: https://www.mdpi.com/2076-3417/13/16/9223]**

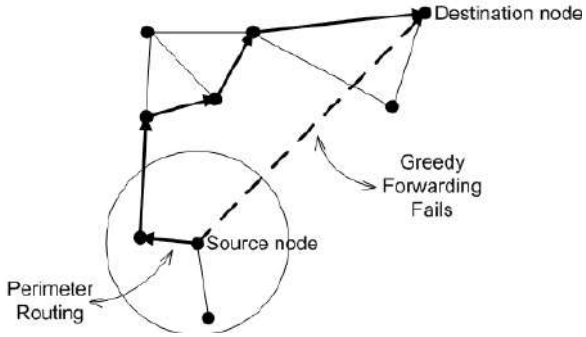


**Figure 4: Greedy Perimeter Stateless Routing [Source:https://www.researchgate.net/figure/Greedy-Perimeter-Stateless-Routing-GPSR_fig3_266216183]**

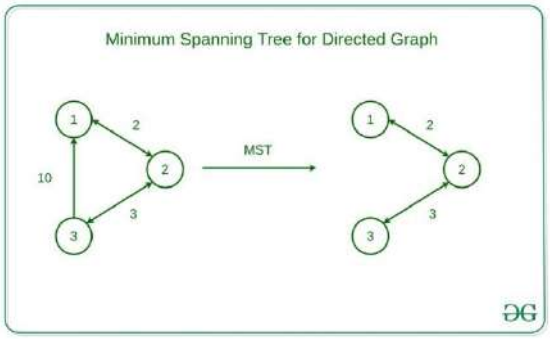


**Figure 5: Minimum Spanning Tree [Source: https://www.geeksforgeeks.org/what-is-minimum-spanning-tree-mst/]**

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**RESEARCH ARTICLE**

# Unlocking Loan Approval : Leveraging Statistical Machine Learning for Informed Decision - Making in Banking

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**ABSTRACT**

The fluctuating nature of individual financial needs often catches people off guard, prompting them to seek monetary assistance, often from banks. However, this surge in loan applications presents a challenge for banks, as they must navigate the delicate balance between meeting customer needs and mitigating risks associated with loan defaults. With an increasing number of loan applicants, banks face the daunting task of allocating their limited resources to individuals who pose the least risk of default. Leveraging the power of statistical machine learning offers a promising solution to this challenge. By harnessing advanced analytical techniques, banks can predict the likelihood of loan repayment and identify safer lending options. Through the development of predictive models, banks can streamline their decision-making process, accurately assessing loan applications to approve or reject them with greater confidence. In essence, by integrating statistical machine learning into their loan approval processes, banks can optimize resource allocation, minimize risk exposure, and ultimately better serve their customers' financial needs.

**Keywords :**Decision tree algorithm, credit risk assessment, Prediction, classification, loan approval

## INTRODUCTION

Banks serve as pivotal financial institutions, crucial for sustaining societal functions. Their primary role lies in providing lending services, which, in today's competitive landscape, demands strategic establishment for survival. Leveraging deposits to extend loans at higher interest rates enables banks to maximize profitability, provided they maintain adequate liquidity to meet customer withdrawal demands. However, the inherent challenge lies in mitigating credit risk, wherein borrowers default on their obligations, leading to bad debts that pose significant risks

to the bank's stability. Effective credit risk assessment becomes imperative to distinguish between defaulters and non-defaulters among loan applicants. Developing a robust model for such assessment is intricate, given the escalating loan demands. Prototypical models employing collaborative machine learning approaches offer a promising solution. By constructing a comprehensive model, banks can make informed decisions regarding loan approval, thus minimizing potential losses and bolstering credit volume. Central to this assessment is the prediction of the Probability of Default (PD) of an applicant, necessitating a multifaceted model that considers various applicant attributes. However, data inconsistencies such as missing values and outliers pose challenges, requiring meticulous handling before model construction. Classification modeling, particularly using Decision Trees, emerges as a potent data mining approach to classify applicants as defaulters or non-defaulters. By integrating these steps into a cohesive model, banks can effectively predict applicant default probabilities, facilitating prudent lending decisions. In summary, by employing advanced data mining techniques and comprehensive risk assessment models, banks can optimize profitability, mitigate credit risks, and ensure prudent lending practices in today's dynamic financial landscape.

Therefore, the study aims to achieve the following objectives:

- Implementing a predictive model tailored for bankers to discern credible loan applicants.
- Evaluating the applicability of the model in aiding financial institutions to make informed decisions regarding loan approvals or rejections.
- Developing a data mining model using R to forecast the Probability of Default (PD) for new loan applicants within a banking context.

## LITERATURE SURVEY

In the article written by Sudhakar, and Reddy (2016) , an efficient prediction model was presented to identify customers applying for bank loans. Utilizing the Decision Tree algorithm, relevant attributes for credibility assessment were predicted, facilitating loan approval decisions for customers. Aboobyda, and Tarig (2016) introduced a model leveraging data from the banking sector to forecast loan statuses. Employing three classification algorithms—j48, bayes Net, and naïve Bayes—the model was implemented and validated using Weka. Notably, the j48 algorithm demonstrated superior accuracy, offering valuable insights for loan decision-making.Kavitha (2016) proposed an enhanced Risk Prediction Clustering Algorithm to assess the status of loan applicants. By integrating Primary and Secondary Levels of Risk assessments and leveraging Association Rule techniques, redundancy was minimized, enhancing the accuracy of loan status predictions. Somayyeh, and Abdolkarim (2015) utilized a decision tree model for classification and genetic algorithms for feature selection. Testing the model with Weka showcased its effectiveness in identifying key features relevant to loan approval decisions. Hussain, and Shorouq (2014) presented two credit scoring models employing data mining techniques tailored for Jordanian commercial banks. Comparative analysis revealed that the logistic regression model outperformed the radical basis function model in terms of accuracy.Blanco et.al (2013) developed non-parametric credit scoring models based on the multilayer perceptron approach, demonstrating superior performance compared to traditional linear discriminant analysis, logistic regression, and quadratic discriminant analysis techniques. Harris (2013) conducted a comparison of support vector machine-based credit-scoring models using Broad and Narrow default definitions. Results indicated that models derived from Broad definition default outperformed those from Narrow definition default. Abhijit, and Chawan (2013) explored bank loan default risk analysis, encompassing various data mining techniques such as Decision Trees, Random Forests, Boosting, Bayes classification, and Bagging algorithms, providing insights into financial data analysis. Francesca (2012) introduced a discrete survival model to assess default risk, providing experimental evidence using the Italian banking system. Seema, and Anjali (2011) tested an integrated model on a dataset from Indian banks, combining Logistic Regression, Radial Basis Neural Network, Multilayer Perceptron Model, Decision Trees, and Support Vector Machine techniques for credit scoring analysis.. Hamid, and Ahmad (2011) estimated the creditworthiness of customers using a Fuzzy Expert System, followed by Data Mining Algorithms via Clementine software. Sudhamathy and Jothi Venkateswaran (2016) proposed a framework to identify the Probability of Default

of a Bank Loan applicant effectively, demonstrating high accuracy and precision in model predictions. Kalyani, Rawate and Tijare (2017) introduced a predictive system for bank loan credibility, applying the Random Forest Data Mining Algorithm to predict relevant attributes for assessing credibility.

**PROPOSED WORK**

The proposed model is centred on accurately predicting the creditworthiness of customers regarding loan repayment, achieved through a comprehensive analysis of various customer attributes. These attributes, reflecting different aspects of customer behavior, serve as inputs to the model. Based on the classifier's output, decisions regarding loan approval or rejection can be confidently made. Utilizing diverse data analytics tools, the model facilitates the forecasting of loan predictions and their associated severity levels. This entails training the data using various algorithms and subsequently comparing user data with the trained dataset to predict loan nature. The R package is employed for data preparation and classification model development. In real-time scenarios, customer datasets often contain missing or imputed data, necessitating the replacement of such data with valid entries derived from available completed data. Furthermore, the dataset encompasses numerous attributes defining customer credibility across various types of loans, with some attribute values potentially exhibiting outliers. It becomes imperative to address these outliers to ensure the dataset's suitability for subsequent modeling.
The steps involved in constructing the model for this study are outlined as follows:

Step 1 – Data Selection
Step 2 – Data Pre-Processing
- Step 2.1 – Missing values replacing
- Step 2.2 – Outlier Detection
- Step 2.3 – Outlier Removal
- Step 2.4 – Imputations Removal
- Step 2.5 – Splitting Training & Test Datasets

Step 3 – Features/Variables Selection
- Step 3.1 – Correlation Analysis of features
- Step 3.2 – Logistic regression analysis
- Step 3.3 – Variables/features Selection

Step 4 – Building Classification Model
Step 5 – Predicting Class Labels of Test Dataset
Step 6 – Evaluating Predictions

The major steps of the credit risk analysis and prediction modeling using R is presented in figure 1. However, evaluating the creditworthiness of applicants is done based on:

**Bank Loan Prediction System:** This application enables banks to assess customers' eligibility for loans efficiently.

**Online Loan Eligibility Check for Individuals:** Users can conveniently determine their loan eligibility online by submitting necessary information. They will receive an email notification indicating their eligibility status, eliminating the need for in-person visits to the bank and enhancing user experience.
The process flow for applicants, from submitting their application to the approval or rejection of the loan, is detailed and illustrated in Figure 2

## RESULTS AND DISCUSSION

**Dataset selection**

1) Data relating to bank loan status has been collected
2) Secondary data was obtained
3) Dataset – "Bank Loan Status Dataset" from Kaggle.com

**Data Pre-Processing**

1) **Outlier Detection:** To detect outliers in the numeric attributes, the values of these attributes are first normalized to fall within the range of [0, 1]. Subsequently, boxplots are generated to visualize and identify any outlier values.
2) **Outliers Removal:** The observations which were out of range (based on the rankings) were removed
3) **Imputations Removal:** The multiple imputation method was used to remove the null values for both numeric and quantitative attributes.
4) **Splitting Training & Test Datasets:** Before proceeding to the further steps, the dataset was split into training and test datasets so that the model can be built using the training dataset. R function was used for this purpose.

**Features Selection:**

1) **Correlation Analysis:**Datasets often include irrelevant or redundant features that can unnecessarily complicate the model. Therefore, eliminating such redundant features can enhance the model's efficiency. Correlation analysis is conducted independently for each data type—numeric and nominal. The results indicate that there is no significant correlation among any of the features, whether numeric or nominal. As a result, no features are removed in this step. The output is presented in Figure 3.
2)**Logistic regression:**Logistic regression analysis was employed to assess the influence of independent variables on the dependent variable. Subsequently, significant variables identified through this analysis were selected for further examination and analysis. The results of logistic regression is tabulated in Table 1.
3) **Variables/ Features selection:**Only the significant variables identified in the analysis were retained for further investigation. Features that did not contribute to prediction were consequently dropped at this stage, streamlining the study to focus solely on pertinent variables.

**Building Model Classification**

Model classification is a fundamental data analysis technique used to predict categorical labels. In this study, we employed the decision tree model to predict the probability of default. The 'rpart()' function was utilized to derive a model from the training dataset. The results of the classification analysis are shown in Table 2. The feature selection threshold is illustrated in Figure 4. Additionally, the classification tree is depicted in Figure 5.
Predicting Class Labels of Test Dataset : Prediction The model is tested using the test dataset by using the predict() function. The results of the predicted classes are presented in Table 3.

**Evaluating Predictions**

Common metrics calculated from the confusion matrix are Precision, Accuracy, TP Rate and FP Rate. From our resultant data we get the values of the above metrics by applying the values as derived below with True Defaults = 88, False Default = 55, Total Default = 1035, True Nondefault = 2910, False Nondefault = 947, Total Nondefault = 2965 and Total Testset = 4000

The calculations for the same are listed below. Also the measures are presented in Table 4.

$$\text{Precision} = \frac{\text{True Defaults}}{\text{True Defaults} + \text{False Defaults}} = 88 / (88+55) = 0.6154$$

$$\text{Accuracy} = \frac{\text{True Defaults} + \text{True Nondefaults}}{\text{Total Testset}} = (88 + 2910) / 4000 = 0.7495$$

$$\text{TP Rate} = \frac{\text{True Defaults}}{\text{Total Defaults}} = 88 / 1035 = 0.085$$

FP Rate = $\frac{\text{False Defaults}}{\text{Total Nondefaults}}$ = 55 / 2965 = 0.0185

These results demonstrate that the proposed model performs with good accuracy and precision, making it suitable for credit scoring applications. A similar analysis can be conducted to assess the likelihood of loan approval or rejection.

## CONCLUSION

This paper presents a framework aimed at enabling banks to conduct effective credit risk assessments, thereby aiding in the avoidance of significant losses. The Probability of Default (PD) was estimated as a crucial metric to assist banks in risk management. Leveraging a Decision Tree model, the framework accurately predicts class labels for new loan applicants. The derived metrics demonstrate the model's high accuracy and precision. This application serves to assist banks in distinguishing between loan defaulters and non-defaulters, facilitating proactive measures based on the identified loan repayment statuses. By enabling timely actions, the framework has the potential to mitigate the occurrence of bad debts, thereby safeguarding banks from substantial financial losses.

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**Table 1. Results of logistic regression Deviance Residuals**

| Minimum | IQ | Median | 3Q | Maximum |
|---|---|---|---|---|
| **-5.6586** | **0.0882** | **0.2199** | **0.2559** | **0.3857** |

| Coefficients | Estimate | Std. Error | t-value | Pr(>\|t\|) |
|---|---|---|---|---|
| **(Intercept)** | 7.431e-01 | 6.029e-03 | 123.258 | <2e-16*** |
| **Current Loan Amount** | -4.557e-04 | 1.092e-04 | -4.173 | 3.01e-05*** |
| **Term** | -8.537e-02 | 3.949e-03 | -21.617 | <2e-16*** |
| **Annual Income** | 2.956e-03 | 1.720e-04 | 17.182 | < 2e-16 *** |
| **Years in current job** | 1.230e-02 | 3.623e-03 | 3.394 | 0.000688 *** |
| **Home Ownership** | 2.690e-02 | 2.583e-03 | 10.416 | < 2e-16 *** |
| **Purpose** | -1.475e-02 | 5.154e-03 | -2.862 | 0.004217 ** |
| **Monthly Debt** | -8.548e-04 | 1.728e-04 | -4.947 | 7.56e-07 *** |
| **Years of Credit History** | 6.806e-04 | 2.460e-04 | 2.767 | 0.005656** |
| **Number of Open Accounts** | -7.111e-04 | 3.581e-04 | -1.986 | 0.047074 * |
| **Number of Credit Problems** | -3.867e-03 | 3.341e-03 | -1.158 | 0.247005 |
| **Current Credit Balance** | -5.099e-06 | 4.185e-04 | -0.012 | 0.990280 |

**Table 2. Results of Classification**

| CP | nsplit | rel error | xerror | xstd |
|---|---|---|---|---|
| 0.002657 | 0 | 1.0000 | 1.0000 | 0.026762 |
| 0.001932 | 7 | 0.97778 | 1.0135 | 0.026878 |
| 0.001546 | 8 | 0.97585 | 1.0290 | 0.027009 |
| 0.000967 | 13 | 0.96812 | 1.0425 | 0.027121 |
| 0.000480 | 15 | 0.96618 | 1.0860 | 0.027504 |
| 0.000193 | 21 | 0.96232 | 1.0908 | 0.027504 |
| 0.000000 | 31 | 0.96039 | 1.0995 | 0.027570 |

**Table 3. Results of Predicted class**

| | Defaulter | Non-defaulter |
|---|---|---|
| **Defaulter** | 88 | 947 |
| **Non-defaulter** | 55 | 2910 |

**Table 4. Measures from the Confusion Matrix**

| Precision | Accuracy | TP rate | FP rate |
|---|---|---|---|
| 0.6154 | 0.7495 | 0.085 | 0.0185 |

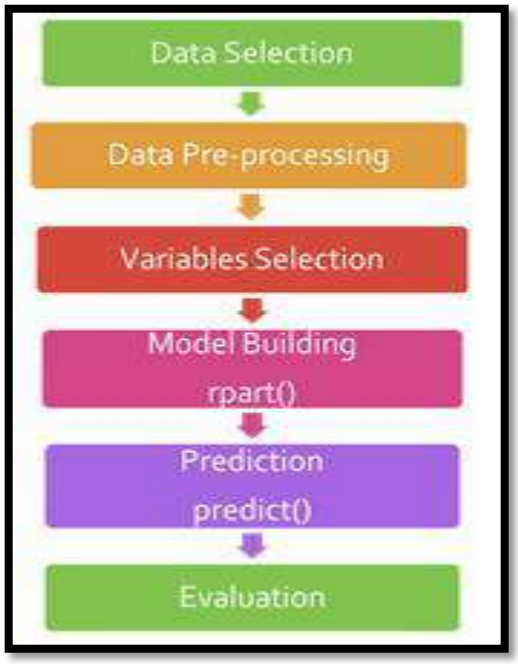


**Figure 1: Major steps of the credit risk analysis and prediction modeling using R**

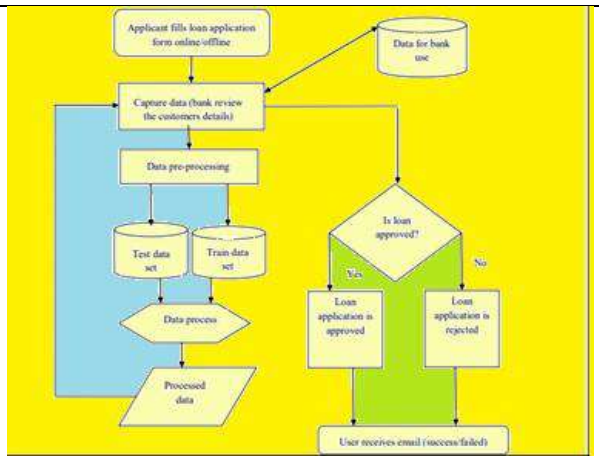


**Figure.2: Process Flow of the Applicants**

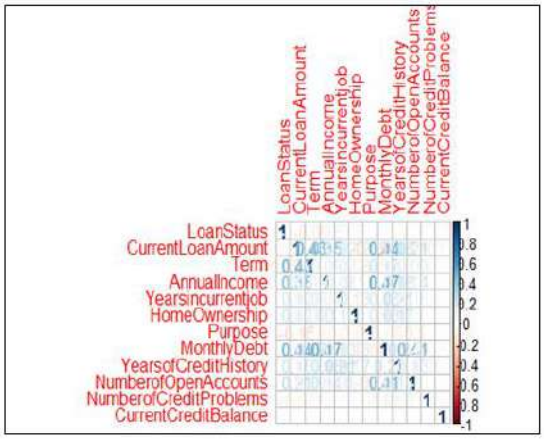


**Figure. 3 Correlation analysis**

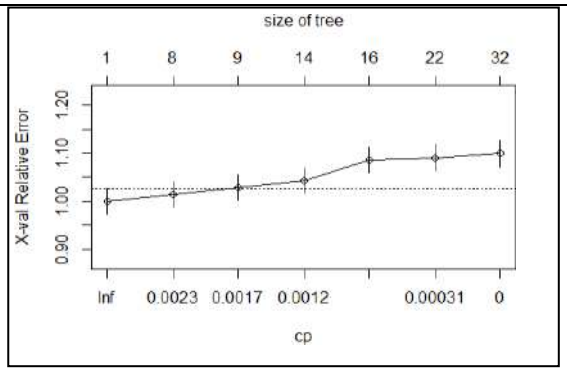


**Figure.4 Threshold for features selection**

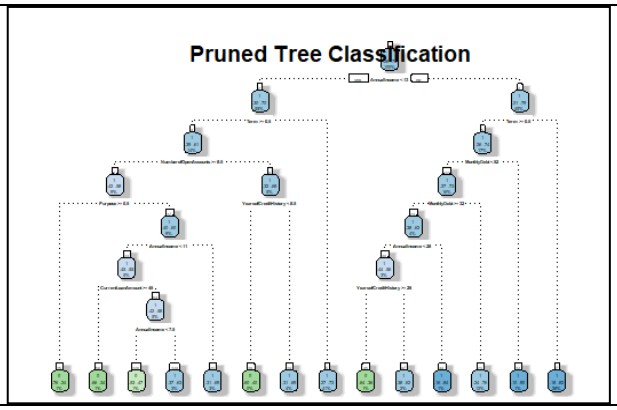


**Figure. 5 Classification tree**

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**RESEARCH ARTICLE**

# Health Status of Tribal Women in Tamilnadu: A Micro Level Study

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**ABSTRACT**

Particular consideration must be given to the health issues in India's indigenous populations. Research studies that are now available indicate that the unique health issues faced by the tribal population are primarily caused by their habitat, which includes challenging topography and biologically varied niches. It has been discovered that the health, nutrition, and medico-genetic issues that various tribal groups face are distinct and pose a significant challenge for which adequate answers must be sought through the planning and development of pertinent research studies. Certain health issues and genetic anomalies, such as sickle cell anemia, a deficiency in the red cell enzyme G-6-PD, and sexually transmitted infections (STDs), are specific to India's primitive tribal populations. Their unhygienic living circumstances, ignorance, poor personal cleanliness, and a lack of health knowledge are the primary causes of their illness. ST populations are still disproportionately affected by "diseases of the poor," such as infectious diseases and malnutrition. Both adult and child populations have shown high levels of chronic under nutrition. Among STs, micronutrient malnutrition, which includes anemia and iodine deficiency illnesses, is also a significant issue. Malaria still exists, especially in tribal communities residing in forested settings, and in certain places it has been reported to be becoming more common. Different tribal populations have different tuberculosis prevalence rates. Therefore, this micro level study analyse health status of tribal women in Pachimali Hills of Tamilnadu

**Keywords:** Schedule Tribes, socio economic development, health status, health problem, health scheme

## INTRODUCTION

There are many different kinds of indigenous people living in India. In India, the Scheduled Tribe population is among the most economically disadvantaged and marginalized communities. India is the country with the largest tribal population in the world, numbering over 10.2 crores. This makes about 8.6% of the nation's overall population [1]. One of the main forces driving change in the direction of progress is education. In actuality, education contributes to the inner strength of tribal communities, which enables them to face new obstacles in life, as well as the economic

prosperity of the tribes. It is an action, a sequence of actions, or a procedure that could either enhance the quality of life right now or expand opportunities for living in the future [2]. It is the single most significant way that people and society can enhance their own endowments, increase their ability, get beyond obstacles, and increase prospects for a long-term development in their well-being. Education has recently been highlighted by Professor Amartya Sen as a crucial factor in any inclusive economic progress. Thus, improving the economic and social circumstances of the Scheduled Tribes can be achieved through education [3].

**Tribal Setting in Tamil Nadu**

In general, Tamil Nadu can be divided into three sections: the eastern coast line region, the middle plain area, and the north and west mountain regions, which are home to the majority of the tribal population. The Jawadhu and Elagiri hills in North Arcot district, the Kalrayan hills in South Arcot district, the Pachamalai, the Kollimalai and Yergadu hills in Salem district, the Anamalai of Coimbator district, the Sitteri hills in Dharmabari district, the Palanimalai of Dindigul district, the Elumalai (cardamom hills) hills, and the Varshanad hills in Theni district are among Tamil Nadu's most significant hills. According to the 1991 census, Tamil Nadu had 5.74 lakh tribal people and 5.58 crore ordinary people living there. Tamil Nadu has a relatively low population concentration of tribal people. The proportion of tribal people to the overall population was 1.03%. The state is home to these 36 tribal tribes.

**Pachamalai Hills**

Pachamalai is situated in the northwestern region of the Tiruchirappalli district, which is divided from the Salem district by the Thuraiyur taluk. In the Malaiyalee (hillmen) district, this is the sole significant hill. At roughly 2000 feet above the mean sea level, the hill range has peaks that reach up to 3400 feet in elevation. This hill is almost 20 kilometers long and shaped like an hourglass. We may get the summit of this hill by taking the Ghat road, which is roughly 31 km long and starts at Shobanaburam, the settlement at the base of the hill. There are eleven hairpin curves in total, and the roads are kept in good condition. A notice board has been erected at the valley view point, three kilometers from the top of the Ghat Road, by the highway division authorities. From there, there is a lovely view of the hill below and the valleys on the slopes.

Two travelers' quest houses—one run by the local Panchayat Union and the other by the forest department—can be found at the top of the hill at the end of the ghat road. The people of Pachamalai Hill (also known as "Green Hill") were somewhat illiterate due to their remote location, which made them fixated on the contemporary medical system. They were dressed traditionally and lived in rudimentary homes in undeveloped villages before colonization. In his guidebook for the Salem district (1887), HizeFaine described the tribal culture, illiteracy, native doctors, daily physical labor, home kinds, and clothing patterns of the people living there. Following independence, the Indian government legitimized their status by enacting several constitutional acts, establishing Directive Principles for State Policies, and implementing numerous social programs to improve their socioeconomic circumstances.

The Tribal Welfare Department, a distinct branch, was founded and given the authority to implement appropriate welfare programs. Guidelines to prioritize tribal areas were also provided to other departments. The Malayalee tribes of Pachamalai Hill experienced socioeconomic and cultural changes as a result of numerous schemes. The hill tribes kept up their use of various tribal languages. As soon as mountains were discovered above the sea's surface, the Tamil race emerged. Tamil languages assert their antiquity, and ancient Tamil literature amply demonstrates that the Tamils discovered one of the original human races. There are a lot of herbal plants on the hill. For the sake of the tribal pupils, there are eight residential schools. For their usage, there are ten public distribution centers, four branch post offices, one government hospital, one veterinary subcenter, and a cooperative society.

The Malayalees are the people who inhabit this hill and are classified as tribal. Part of the Uppiliyaburam Assembly seat, which has been set aside for the return of a Scheduled Tribe member to the Tamil Nadu assembly, includes Pachamalai. Most Keralans live in poverty. Only Malayalees are allowed to live in the Pachamalai highlands; others are not permitted to migrate here. Individuals in this place appear to live in harmony and cooperation with one

another. This is demonstrated by the fact that the presidents and ward members have always been chosen unanimously and without opposition in previous elections.

**Empirical Literature**

In actuality, education contributes to the inner power of tribal societies, which enables them to face new obstacles in life, as well as the economic development of the tribes [4]. Among India's backward communities, literacy and educational attainment are strong markers of social and economic growth [5]. In terms of literacy and education, the tribes currently trail behind both the general population and the Scheduled Caste community. Women from Scheduled Tribes, who have the lowest rates of literacy in the nation, are particularly more affected by this inequality [6]. Among the scheduled tribes, there is a notable disparity between male and female educational attainment and literacy rates. Education—particularly primary education—is regarded as being extremely vital for the overall development of tribal groups and is especially useful in fostering the confidence of the tribes to interact with outsiders on an equal footing [7].

The scheduled tribes are still far behind in practically every conventional development criteria, even with the government's earnest and coordinated efforts to support their overall development [8]. Due to their lack of awareness of the majority of programs and policies designed for their advancement, they are unable to take part in the development process [9]. This is mostly because indigenous people have a relatively low level of education and a high incidence of illiteracy. Therefore, the scheduled tribes' educational standing and the government's role in promoting this are crucial [10]. It is often recognized that, in comparison to the general population, tribes have highly poor educational backgrounds. Thus, improving the economic and social circumstances of the Scheduled Tribes can be achieved through education [11].

Different tribal groups have been shown to have distinct health, nutritional, and medico-genetic issues. These issues pose a significant task, for which adequate solutions must be sought through the planning and development of pertinent research projects [12]. Certain health issues and genetic anomalies, such as sickle cell anemia, a deficiency in the red cell enzyme G-6-PD, and sexually transmitted infections (STDs), are specific to India's primitive tribal populations. The primary causes of their poor health include unhygienic environments, ignorance, a lack of personal hygiene routine, and a lack of health education [13]. ST populations are still disproportionately affected by "diseases of the poor," such as infectious diseases and malnutrition. Both adult and child populations have high rates of chronic undernutrition [14].

Among STs, micronutrient malnutrition, which includes anemia and iodine deficiency illnesses, is also a significant issue. Malaria still exists, especially among tribal communities residing in forested areas, and in certain places it has been reported to be becoming more common [15]. Different tribal populations have different tuberculosis prevalence rates. Numerous studies have revealed that the prevalence and patterns of tuberculosis (TB) in ST communities do not differ significantly from those in non-ST communities. However, due to the challenging terrain and restricted medicine supply in many tribal locations, ST-specific TB control strategies require special attention [16]. While the prevalence rates of HIV/AIDS among some tribal groups remain low due to their geographic isolation and limited interactions with other communities, in certain areas STs are becoming a high-risk group for HIV/AIDS due to their migration for employment opportunities or displacement [17].

## RESEARCH METHODS

Primary sources were used as the data gathering sources for this study. In the study areas, interviews, both semi-structured and structured, were carried out with the tribal households. Interviewees were asked, via a carefully designed questionnaire, how they perceived the health conditions. The current study is being carried out in Tamilnadu'sPachamalai Hills in the Tiruchirappalli district. The chosen study. Using a stratification process based on the tribal population, the villages are chosen based on the higher concentration of tribal households. Tribal villages in

Mayampady and Nallamathiwere chosen for the study. Eighty sample tribal households were picked from Mayampady Village and eighty sample tribal households were recruited from Nallamathivillage. For a comprehensive analysis, 160 sample tribal homes from the villages of Mayampady and Nallamathiwere chosen.

**Data Analysis and Results**

Particular consideration must be given to the health issues in India's indigenous populations. Research studies that are now available indicate that the unique health issues faced by the tribal population are primarily caused by their habitat, which includes challenging topography and biologically varied niches.

**Status of Immunizations**

According to Table 1.1, in both study regions' villages, 31.25 percent and 68.75 percent of children aged 0 to 5 have received all recommended vaccinations and none at all. Furthermore, in the Mayampady and NallamathiVillages, 32.50 percent and 30.00 percent of children aged 0 to 5 have received all recommended vaccinations, whereas 67.50 percent and 70.00 percent of children in the same age range have not received all recommended vaccinations. It has been noted that the majority of children in the research areas are not fully immunized, which could lead to various health issues such as physical disabilities, mental disorders, and other illnesses.

**Reproductive Age**

According to Table 1.2, of the 90 sample tribes, 68.75 percent of them married between the ages of 15 and 18; 12.23 percent married between the ages of 19 and above; and 11% married women in both age categories below the age of 15. Of the 45 sample tribal women, 28 and 29 (62.22 and 64.44 percent) were married between the ages of 15 and 18; the remaining 17.92 and 15.25 percent were married after the age of 19, and the remaining 12 and 11 percent were married before the age of 15 in the study area of Mayampady and Nallamathi Villages. It is noted that the majority of the study's tribal women were married before turning eighteen.

**Diseased Persons - Gender Distribution**

Table 1 shows the distribution of diseased people in the sample area by gender.According to Figure 3, out of 160 sample tribes in Mayampady and NallamathiVillages, the afflicted individuals are 35 and 38. Of these, 62.86 and 68.42 percent are male and 37.14 and 31.58 percent are female. In the studied locations, it has been noted that male tribes account for the majority of sickness cases.

**Diseased Persons-age wise**

According to Table 1.4, in the Mayampady and NallamathiVillages, 80.92 percent and 78.99 percent of the sick individuals are in the productive age range of 15 to 50, while the remaining 16.79 percent and 18.11 percent are in the unproductive age group of up to 14 years old. In the age range of above 50, the prevalence of disease is extremely low. Their size in relation to the overall population is the reason, not their excellent health.

**Affected Diseases**

Table 1.5 shows how the sample tribes are distributed among the study area's affected diseases. In the Mayampady and NallamathiVillages, 60.00 percent and 65.71 percent of the afflicted individuals suffer from fever, while 28.57 percent and 31.43% from malaria and 11.43 percent from dengue.

**Consults the Doctor**

Table 1.6 shows that, in the study region of Mayampady and NallamathiVillages, 74.29% and 80.00% of the unwell individuals see a doctor, while 25.71 percent and 20.00 percent do not see a doctor for treatment.

**Expenditure for Their Diseases**

Table 1.7 shows that, in the Mayampady and NallamathiVillages, approximately 44.44 percent and 46.67 percent of the sick individuals spend less than Rs. 1,000 on their treatment, 17.78 percent and 15.56 percent spend between Rs. 1001 and Rs. 2000, 11.11 percent and 13.33 percent spend between Rs. 2,001 and Rs. 3,000, and 4.44 percent and 8.89 percent spend more than Rs. 3000.

**Government Medical Services**

The sample tribes' opinions on government medical services are explained in Table 1.8. In the study regions of Mayampady and NallamathiVillages, it was noted that 38.75 percent and 36.88 percent of the households, respectively, expressed the opinion that they had a visit from the medical officer. Furthermore, 42.50% and 393.8% of respondents believe that the government health clinics provide them with adequate care. Regarding medication, 42.50 percent and 41.25 percent of respondents believe that the government health centers provide them with enough medication. It is noted that the government health centers in the villages of Mayampady and Nallamathiare operating satisfactorily.

**Awareness about the AIDS**

According to Table 1.9, 36.25 percent and 39.37 percent have heard of the illness, and 26.25 percent and 30.00 percent are aware of its causes and symptoms. It has been noted that the government needs to implement more AIDS awareness programs because approximately 45 percent of families are unaware of the disease's causes and symptoms.

**Distance to the Medical Center**

In the Mayampady and NallamathiVillages, Table 1.10 looks at 18.75% and 24.37% of the homes that don't have a medical center within 16 km, and 81.25 percent and 75.63% of the houses that are closer to medical centers at a radius of 10 km. It might have a detrimental impact on the health of the homes in the Nellore District research locations.

## SYNOPSIS OF RESULTS

From the foregoing discussion, it may be inferred that the Immunization Status of tribes shows that around 31.25% of children aged 0 to 5 have received all recommended vaccinations, while 68.75% have not. It has been noted that the majority of children in the research areas are not fully immunized, which could lead to various health issues such as physical disabilities, mental disorders, and other illnesses. Among the reproductive age group of women, approximately 68.75 percent were married between the ages of 15 and 18; 12.23 percent were married at 19 or older; and 11% were married when they were under the age of 15. It is noted that the majority of the study's tribal women were married before turning eighteen. Males are more likely than females to suffer from diseases, and those in the productive age group are more likely to do so (65.71%). The two main illnesses impacting the sample population are fever and malaria.

In the research region of Mayampady and NallamathiVillages, it is predominant that 74.29 percent and 80.00 percent of the affected consult a doctor, respectively, whereas 25.71 percent and 20.00 percent do not seek medical attention. From a health perspective, nearly everyone is aware of the causes, symptoms, and implications of AIDS. The results indicate that a very tiny proportion of respondents receive government support, and this is likely due to the fact that most tribes were unaware of this program. It has been determined that the health, dietary, and medical-genetic issues faced by various ethnic groups are distinct and pose a significant challenge for which suitable solutions must be developed.

## CONCLUSION

It is possible to draw the conclusion that, in the interior hill tracts where the tribes primarily live, the government should give special consideration to the construction of more extensive basic infrastructural facilities on a priority basis. The aboriginal population has relatively limited access to current health programs and rights, such as those related to medications and bed services. In order to reduce the issue, the fluoride-affected tribes must also receive generous help in the form of free medications and an increase in their ration quota to ensure that they eat the right foods. The issue has been there for many years after independence, so both the state and district authorities need to give it careful thought. The problem is also being caused by the state government's incompetence, which includes giving some districts one-way financing while ignoring other districts. The tribes would be able to exercise their "right to health" if a specific quota under the Aarogya Sri scheme—the state health program for all middle-class and lower-class Andhra Pradesh residents—is added to those living in fluorosis-affected areas.

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**Table 1. Child Immunization Status**

| **Child Immunization** | Mayampady Village | NallamathiVillage | **Total** |
|---|---|---|---|
| fully immunized | 26(32.50) | 28(30.00) | 54(31.25) |
| Not fully immunized | 54(67.50) | 56(70.00) | 110(68.75) |
| **Total** | **80(100.00)** | **80(100.00)** | **160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the per centage to total

**Table.2Reproductive Age of Women**

| **Reproductive Age of Women** | Mayampady Village | Nallamathi Village | **Total** |
|---|---|---|---|
| < 15 | 05(11.11) | 06(13.33) | 11(12.22) |
| 15-18 | 28(62.22) | 29(64.44) | 57(63.33) |
| 19 and above | 12(26.67) | 10(22.21) | 22(24.45) |
| **Total** | **45/80(100.00)** | **45/80(100.00)** | **90/160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 3Diseased Persons (Gender-wise)**

| **Diseased Persons (Gender-wise)** | Mayampady Village | Nallamathi Village | **Total** |
|---|---|---|---|
| male | 22(62.86) | 26(68.42) | 48(65.75) |
| female | 13(37.14) | 12(31.58) | 25(34.25) |
| **Total** | **35/80(100.00)** | **38/80(100.00)** | **73/160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 4Diseased Persons (Age wise)**

| **Diseased Persons (Age wise)** | Mayampady Village | Nallamathi Village | **Total** |
|---|---|---|---|
| Up to 14 | 2(5.71) | 3(8.57) | 4(6.85) |
| 15-50 | 26(74.28) | 27(77.14) | 53(72.60) |
| 50 and above | 7(20.00) | 8(22.86) | 15(20.55) |
| **Total** | **35/80(100.00)** | **38/80(100.00)** | **73/160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the per centage to total

**Table 5 sample tribes according affected diseases**

| **Type of disease** | Mayampady Village | Nallamathi Village | **Total** |
|---|---|---|---|
| Fever | 21(60.00) | 23(65.71) | 45(60.27) |
| Malaria | 10(28.57) | 11(31.43) | 21(28.77) |
| Dengue | 4(11.43) | 4(11.43) | 8(10.96) |
| **Total** | **35/80(100.00)** | **38/80(100.00)** | **73/160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 6 Diseased tribes whether consults the Doctor for Their Treatment**

| Opinion of diseased persons | Mayampady Village | Nallamathi Village | Total |
|---|---|---|---|
| Yes | 26(74.29) | 28(80.00) | 53(72.60) |
| No | 9(25.71) | 10(20.00) | 19(20.03) |
| **Total** | **35/80(100.00)** | **38/80(100.00)** | **73/160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 7 Diseased** tribes **According To Their Expenditure for Their Diseases**

| Type of disease | Mayampady Village | Nallamathi Village | Total |
|---|---|---|---|
| Below 1,000 | 20(44.44) | 21(46.67) | 45(45.56) |
| 1,001-2,000 | 8(17.78) | 7(15.56) | 15(16.67) |
| 2,001-3,000 | 5(11.11) | 6(13.33) | 11(12.22) |
| 3,001 and above | 2(4.44) | 4(8.89) | 6(6.67) |
| **Total** | **35/80(100.00)** | **38/80(100.00)** | **73/160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 8 Opinion of the Sample tribes regarding to the Government Medical Services**

| Name of the services | Mayampady Villages | | Nallamathi Villages | | Total |
|---|---|---|---|---|---|
| | Yes | No | Yes | No | |
| Visit of the medical officer | 62(38.75) | 18(11.25) | 59(36.88) | 21(13.12) | 160(100.00) |
| Sufficient Services provided | 68(42.50) | 12 (7.50) | 63(39.38) | 17(10.62) | 160(100.00) |
| Sufficient Medicine provided | 66(41.25) | 14 (8.75) | 67(41.88) | 13(8.12) | 160(100.00) |
| Un necessary tests | 69(43.13) | 11(6.87) | 68(42.50) | 12(7.50) | 160(100.00) |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 9 Awareness about the AIDS in the Sample tribes**

| Awareness about the AIDS | Mayampady Villages | | Nallamathi Villages | | Total |
|---|---|---|---|---|---|
| | Yes | No | Yes | No | |
| Heard about AIDS | 58(36.25) | 22(13.75) | 63(39.37) | 17(10.63) | 160(100.00) |
| Causes | 42(26.25) | 38(23.75) | 48(30.00) | 32(20.00) | 160(100.00) |
| Symptoms | 42(26.25) | 38(23.75) | 43(26.87) | 37(23.13) | 160(100.00) |

Source: Computed Note: Figures in Parentheses represents the percentage to total

**Table 10 distance to the Medical Center to the Sample tribes**

| | Mayampady Villages | Nallamathi Villages |
|---|---|---|
| **Distance in km** | **No of persons** | **No of Persons** |
| 10 | 130(81.25) | 121(75.63) |
| 16 | 30(18.75) | 39(24.37) |
| **Total** | **160(100.00)** | **160(100.00)** |

Source: Computed Note: Figures in Parentheses represents the percentage to total

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**RESEARCH ARTICLE**

# Coprime Edge Labeling of Graphs

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## ABSTRACT

The purpose of this paper is to open up new directions for learning in the field of graph labeling. In this paper, some new graph labeling techniques namelyrelatively prime edge labeling and minimum coprime edge labelingare introduced. In prime labeling, vertices are labeled in such a way that, any two adjacent vertices have relatively prime labels, whereas in relatively prime edge labeling edges are labeled in such a way that any two adjacent edges have relatively prime labels. Coprime labeling maintains the same criterion as prime labeling with adjacent vertices using any set of distinct positive integers. The minimum value m for which G has Coprime labeling is defined as a minimum coprime number,$p\tau(G)$. Asan extension of the above definition for edges, coprime edge labeling is introduced. We then definethe minimum coprime edge number, $p\tau_E(G)$ to be the minimum value of k for which G has a coprime edge labeling. Finally, the minimum coprime edge number of some graphs is discussed in this paper.

**Keywords**: prime labeling, relatively prime edge labeling, coprime labeling, coprime edge labeling, relatively prime index.
**Mathematics Subject Classification:** 2020: 05C76, 05C78

# INTRODUCTION

The fundamental concepts of Combinatorics and number theory are used in the majority of graph labeling techniques. In number theory, prime number theorem is one the greatest achievement of mathematicians. It provides a way to count the number of primes less than or equal to n and is denoted by $\pi(n)$[6]. In recent years, Graph labeling acts as a significant topic in the field of research. To face the wide range of application challenges, various labeling techniques have been developed. Tout et al. [12] investigated Entringer's concept of prime labeling, which was proposed by Entringer. Many researchers have studied and introduced variousprime graphs, such as the edge vertex prime graph by R. Jagadesh and J.BaskarBabujee [5], the K-Prime graph by S.K. Vaidya and U.M. Prajapati [14], the edge-prime graph concept by Wai-chee-Shiu et al. [12], the SD-Prime graph concept byG.C.Lau and W.C.Shiu [9]. While considering edge labeling, edge pair sum labelingof some Cartesian product of graphs is discussed in [7].In a prime graph, the vertices of G are labeled from 1 to n such that, the labels ofadjacent vertices are relatively prime [3]. As a continuation of the above work foredges, a new labeling technique known as relatively prime edge labeling for a simplegraph is introduced. A graph that admits relatively prime edge labeling is called asrelatively prime edge labeled graph.Coprime labeling came from prime labeling where prime labeling uses the labelsfrom 1 to n, but in coprime labeling the labels have no bound[1]. In coprime labeling, if the vertices are labeled from 1 to k, then the least k is the minimum coprimenumber of G. Motivated by the above work, in this paper, coprime edge labeling of a simple graphis introduced. That is, coprime edge labeling came into use when a graph fails topossess relatively prime edge labeling. That is, for relatively prime edge labelingthe edges uses the labels from 1 to q, whereas in coprime edges labeling the labelsof edges have no bound.

**Preliminaries**

In this section, some basic definitions needed for this study is discussed. Let $G = (V, E)$ be a graph. A bijection $f : V \rightarrow \{1,2,3 \ldots \ldots, |v|\}$ is called prime labeling if for each edge$e \in E$, we have$GCD\,(f(u), f(v)) = 1$. A graph which admits a prime labeling is called a prime graph [4]. Asplund and Fox emphasis on determining the minimal coprime number for graphs that have not been a prime graph [8]. In particular, a coprime labeling of G is a labeling of the vertices of G with distinct integers from the set $\{1, 2, \ldots, k\}$, for some $k \geq n$, in such a way that the labels of any two adjacent vertices are relatively prime. Also, the minimum coprime number $p\tau(G)$ to be the minimum value of k for which G has a coprime labeling[10]. The corresponding labeling of G is called a minimal coprime labeling of G.If $p\tau(G) = n$ , then a corresponding minimal coprime labeling of G is called a prime labeling of G and we call G prime. In [8]John Asplund et.al found the coprime number of complete graph and wheel to be $p_{n-1}$ and $n + 2$ , respectively. In [10] the minimum coprime number of the sum of path and cycles were found.

In [2] the union of two simple graphs $G \cup H$, where $G = (V_1, E_1)$ and $H = (V_2, E_2)$ is the simple graph with vertex set $V_1 \cup V_2$ and edge set $E_1 \cup E_2$. Also, the sum of two graphs G and H, denoted by G + H, is the graph obtained by taking disjoint copies of G and H and then adding every edge $xy$, where $x \in V(G)$ and $y \in V(H)$. The corona product of two graphs G and H is defined as the graph obtained by taking one copy of G and $|V(G)|$ copies of H and joining the $i^{th}$vertex of G to every vertex in the $i^{th}$ copy of H[11].

**Minimum Coprime Edge Labeling**

Throughout this paper, we let $p_i$to be the ith prime number. In this section, thedefinitions of relatively prime edge labeling and coprime edge labeling with someexamples are explained. We then also, examine some theorems related to the co-prime edge labeled graph and find the minimum coprime edge number of a tree.

**Definition 3.1** For a graph, $G = (p, q)$relatively prime edge labeling is defined to be a bijection $f : E \rightarrow \{1,2,3 \ldots \ldots q\}$such that, for each vertex $v \in V(G)$,the labels of the edges incident on $v$ are pairwise relatively prime. A graph that admits a relatively prime edge labeling is called a relatively prime edge labeled graph.

In other words, a graph on p vertices and q edges is said to admit relatively prime edge labeling if we label its edges with the first q natural numbers such that anytwo adjacent edges have relatively prime labels.

**Example 3.2** The relatively prime edge labeling of $C_3$is illustrated below.

**Definition 3.3** For a graph, $G = (p, q)$, coprime edge labeling is defined to be a bijection $f: E \to \{1, 2, \ldots, k\}$such that, for $k \geq q$, and for each vertex $v \in V$,the labels of the edges incident on $v$ are pairwise relatively prime. The minimum value of $k$, for which G is coprime edge labeling is called as minimum coprime edge labeling, with minimum coprime edge number, $p\tau_E(G) = k$. If$p\tau_E(G) = q$, then a corresponding minimum coprime edge labeling of G is called a relatively prime edge labeling.

**Example 3.4** The coprime edge labeling of $K_5$is illustrated below.
The following theorem tells us that every relatively prime edge labeled graph is a coprime edge labeled graph, but the converse fails.

**Theorem3.5** Every relatively prime edge labeled graph is a minimum coprime edge labeled graph, but the converse is not true.
**Proof.** Let $G = (p, q)$ be a relatively prime edge labeled graph. From the definition of relatively prime edge labeling, the edges of G are assigned with labels 1 to q, which is also a minimum coprime edge labeling with minimum coprime number $q$. Hence every relatively prime edge labeled graph is a minimum coprime edge labeled graph. Conversely, in minimum coprime edges labeling the edges of G are assigned with labels 1 to k, but not necessarily q. Thus, every minimum coprime edge labeled graph need not be a relatively prime edge labeled graph. The next theorem finds the minimum coprime edge number of the tree with $p$ vertices and $p - 1$ edges.

**Theorem3.6** If $G = (p, p - 1)$ is a connected acyclic graph with exactly one vertex of degree $p - 1, p > 4$, then G is a coprime edge labeled graph with $p\tau_E(G) = p_{p-2}$
Proof. Suppose G is a not a coprime edge labeled graph for $p > 4$, then G will be a relatively prime edge labeled graph. That is, for each vertex inG, the labels of edges incident on v are pairwise relatively prime. Let w be the vertex in G with degree $p - 1$. Therefore, the labels of edges incident on w are pairwise relatively prime, which is a contradiction. Since the label of edges incident on w will be 1,2,3 ... ... $p - 1$. Hence, G is a coprime edge labeled graph. We now claim that, $p\tau_E(G) = p_{p-2}$.
By labeling the edges of G with $1, 2, 3, 5, 7, \ldots, p_{p-2}$results in a coprime edge labeled graph with minimum coprime edge number $p_{p-2}$. □

**Minimum Coprime Edge Labeling of Complete Graph**
It is clear that, the [2] complete graph $K_p$has no relatively prime edge labeling for $p > 3$. Hence for $p > 3$, one can find the minimum values used to label the edges of complete graph. We use the term minimal coprime edge number, $p\tau_E(G)$to denote the minimum values used to label the edges for coprime edge labeling.
Using computer check, the minimal coprime edge number for $K_p$for $4 \leq p \leq 10$ is given below.

| $p$ | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|
| $p\tau_E(K_p)$ | 7 | 17 | 29 | 47 | 67 | 101 | 127 |

The following Fig 4, shows the minimal coprime edge number of $K_9$
**Observation 4.1**By analyzing the minimum coprime edge labelings constructed for $K_p$, the following facts is verified for $p \leq 10$ :

- All primes up to $p\tau_E(K_p)$are used in minimal coprime edge labeling.
- Minimum coprime edge number $p\tau_E(K_p) \leq p_{\pi(p)+q-p}$
- Minimum coprime edge labeling of $K_p$contains the label from 1 to $p$.

The next theorem shows the upper bound for minimum coprime edge number of the complete graph.

**Theorem 4 .2**For complete graph $K_p$ , $p\tau_E(K_p) \leq p_{[\pi(p)+q-p]}$ , equality holds only when $p = 4$.
Proof
Let $G = (p, q)$be a complete graph with p vertices and q edges.

**Case 1:** For $p = 4$
The number of edges in $K_4$is 6. The edges of $K_4$can be divided into two parts and labeled in such a way that, the outer cycle$C_4$with label $1, 2, 3, 4$ and the remaining edges, that is the non-adjacent edges of $C_4$as $5, 7$. And it is clear that, the above mentioned labeling is coprime edge labeling. Therefore, $p\tau_E(K_4) = 7 = p_4 = p_{[\pi(4)+6-4]}$.
Case **2:** For $p > 4$
Define a labeling function $f: E \to \{1, 2, \cdots, k\}$ , such that $f(v_i v_{i+1}) = i, for\ i = 1, 2, \ldots p-1$ , $f(v_p v_1) = p$ and the remaining $q - p$ edges are labeled with $q - p - 1$ distinct prime numbers and an edge with a non-prime number. We now claim that, the above defined labeling is a coprime edge labeling and there exists an edge $v_i v_k$ , $for\ k \neq i + 1$ labeled with a non –prime number.

Let the edge $v_2 v_5$be labeled with an odd multiple of 3. Now, for each vertex $v_i, i \neq k, 2, 5$ the edges incident on $v_i\ are\ v_i v_k$ , $k = 1,2, \ldots, p$ have the labels $i - 1, i\ and$ the remaining edges are labeled with prime numbers. And for the vertex $v_2$, $v_5$, the label of the edges incident on them are $\{1, 2, odd\ multiple\ of\ 3, p_i's\}$ $and$ $\{4, 5, odd\ multiple\ of\ 3, p_i's\}$. Hence, the label of the edges incident on each vertex of the complete graph are relatively prime. Therefore, the above defined labeling is a coprime edge labeling.

Finally, to find the minimum coprime edge number, it is enough to find the minimumnumber of primes used to label the edges of the complete graph. Hence, the number of primes used to label the edges of $v_1 v_2$, $v_2 v_3$, $\cdots\cdots, v_p v_1$ are $\pi(p)$and the remaining edges $q - p$ are labeled with maximum$q - p - 1$ distinct primes.
Thus,$p\tau_E(K_p) \leq p_{[\pi(p)+q-p-1]}$. That is,$p\tau_E(K_p) < p_{[\pi(p)+q-p]}$ , $for\ p > 4$.
Hence,$p\tau_E(K_p) \leq p_{[\pi(p)+q-p]}$, $p \geq 4$
The following theorems explain the minimum coprime edge number of complete bipartite graph $K_{2,n}$ , $K_{3,n}$and corona product of $K_n$and $K_1$.

**Theorem 4.3** For n > 2,the minimum coprime edge number of the complete bipartite graph $K_{2,n}$ , is $p\tau_E(K_{2,n}) = p_{[2n-3]}$
**Proof**
Let the vertices of $K_{2,n}$are $v_1, v_2, u_1, u_2, \ldots, u_n$.
Define a labeling function $f: E \to \{1, 2, \cdots, k\}$ such that,
$f(v_1 u_1) = 1, f(v_1 u_i) = p_{i-1}, i = 2, \ldots, n, f(v_2 u_i) = p_{n+i-3}$ , $i = 3,4, \ldots, n$ , $f(v_2 u_1) = 4, f(v_2 u_2) = 9$ where $p_i$denotes the i$^{th}$ prime number. (Fig 6)
As the vertices $v_1\ and\ v_2$are of degree n, the labels incident on $v_1\ and\ v_2$are $1, 2, 3, 5, \ldots, p_{n-1}$ and $4, 9, p_n, p_{n+1}, \ldots, p_{2n-3}$ respectively, which are relatively prime.
And also, the each vertex $u_1, u_2, \ldots, u_n$is of degree 2, then the labels incident on $u_1, u_2, \ldots, u_n$are as follows:
$\{1, 4\}, \{p_1, 9\}, \{p_2, p_n\}, \ldots \{p_{n-1}, p_{2n-3}\}$ which are relatively prime. Hence the minimum coprime edge number of $K_{2,n}$ is $p_{2n-3}$.

**Theorem 4.4** For n ≥4,the minimum coprime edge number of the complete bipartite graph $K_{3,n}$ , is $p\tau_E(K_{3,n}) = p_{3n-5}$.
**Proof**
Let the vertices of $K_{3,n}$are $v_1, v_2, v_3, u_1, u_2, \ldots, u_n$.
Define a labeling function $f: E \to \{1, 2, \cdots, k\}$ such that,$f(v_1 u_1) = 1, f(v_1 u_i) = p_{i-1}, i = 2, \ldots, n$ , $f(v_2 u_i) = p_{n+i-3}$ , $i = 3,4, \ldots, n$ , $f(v_2 u_1) = 4, f(v_2 u_2) = 9$ and $f(v_3 u_i) = p_{2n+i-5}$ , $i = 4, 5, \ldots, n$ , $f(v_3 u_1) = 15, f(v_3 u_2) = p_{2n-2}$ , $f(v_3 u_3) = 16$ where $p_i$denotes the i$^{th}$ prime number (Fig 7)

As $v_1, v_2, v_3$is of degree n, the labels incident on $v_1, v_2, v_3$are $\{1, 2, 3, 5, \dots, p_{n-1}\}$ , $\{4, 9, p_n, p_{n+1}, \dots, p_{2n-3}\}$ *and* $\{15, p_{2n-2}, 16, p_{2n-1}, p_{2n}, \dots p_{3n-5}\}$ respectively, which are relatively prime.
And also, the each vertex $u_1, u_2, \dots, u_n$ is of degree 3, then the labels incident on $u_1, u_2, \dots, u_n$are as follows: $\{1, 4, 15\}, \{p_1, 9, p_{2n-2}\}, \{p_2, p_n, 16\}, \dots \{p_{n-1}, p_{2n-3}, p_{3n-5}\}$ which are relatively prime. Hence the minimum coprime edge number of $K_{3,n}$ is $p_{3n-5}$. □

**Theorem 4.5** For $n > 3$, $p\tau_E(K_n \odot K_1) \leq p_{\left[\pi(2n)+\frac{n(n-3)}{2}\right]}$, equality holds only when $n = 4, 5$

**Proof**
We now divide the proof into 3 cases. Let $V(K_n \odot K_1) = \{v_1, v_2, \cdots\cdots, v_n\} \cup \{u_1, u_2, \cdots\cdots, u_n\}$ be the vertices of $K_n \odot K_1$ and the edges are$E(K_n \odot K_1) = E[K_n] \cup \{v_i u_i\}$, for all $i = 1, 2, \dots, n$.
Case 1: For $n = 4$
Define a labeling function, $f: E(K_4 \odot K_1) \to \{1, 2, \cdots, k\}$, such that
$f(v_i u_i) = 2i, i = 1, 2, 3, 4$
$f(v_{i-1} v_i) = 2i - 1, i = 1, 2, 3, 4$ *with* $v_0 = v_4$
$f(v_i v_{i+1}) = 2i + 1, i = 1, 2, 3, 4$ *with* $v_1 = v_5$and the remaining 2 edges are labeled with distinct prime numbers$\{11, 13\}$. Hence the total number of prime numbers assigned to label the edges of $K_4 \odot K_1$are$\pi(8) + 2 = 6$. That is, $p\tau_E(K_4 \odot K_1) = p_6$.(Fig 8)

Case 2: For $n = 5$
Define a labeling function, $f: E(K_5 \odot K_1) \to \{1, 2, \cdots, k\}$, such that
$f(v_i u_i) = 2i, i = 1, 2, 3, 4, 5$
$f(v_{i-1} v_i) = 2i - 1, i = 1, 2, 3, 4, 5$ *with* $v_0 = v_5$
$f(v_i v_{i+1}) = 2i + 1, i = 1, 2, 3, 4, 5$ *with* $v_1 = v_6$and the remaining 9 edges are labeled with distinct prime numbers. Hence the total number of prime numbers assigned to label the edges of $K_5 \odot K_1$are$\pi(10) + 9 = 13$. That is, $p\tau_E(K_5 \odot K_1) = p_{13}$.(Fig 9)

Case 3: For $n > 5$
Define a labeling function, $f: E(K_n \odot K_1) \to \{1, 2, \cdots, k\}$, such that
$f(v_i u_i) = 2i, i = 1, 2, \dots, n$
$f(v_{i-1} v_i) = 2i - 1, i = 1, 2, \dots, n$ *with* $v_0 = v_n$
$f(v_i v_{i+1}) = 2i + 1, i = 1, 2, \dots, n$ *with* $v_1 = v_{n+1}$

We now claim thatthe remaining $\frac{n(n-3)}{2}$ edges are labeled in such a way that atleast 1 edge having a non-prime number. For that, label the edge$v_1 v_4$ as 25 or 55 or so on. (Fig 10)
In this case, the total number of prime numbers assigned to label the edges of $K_n \odot K_1$are$\pi(2n) + \frac{n(n-3)}{2} - 1$. Hence,$p\tau_E(K_n \odot K_1) \leq p_{\left[\pi(2n)+\frac{n(n-3)}{2}\right]}$.
The sum of two graphs$P_2$and $P_n$ is the graph obtained by taking disjoint copies of $P_2$and $P_n$ and then adding every edge$v_i v_j$, where $v_i \in V(P_2)$ and$v_j \in V(P_n)$ [11]. The following theorem finds the minimum coprime edge number of $P_2 + P_n$.

**Theorem 4.6** For $n + 2 \equiv 0(mod\ 3)$, the minimum coprime edge number of $P_2 + P_n$ with $n > 2$ *is*, $p\tau_E(P_2 + P_n) \leq p_{\pi(n+2)+[2n-3]}$

**Proof**
Let $V(P_2 + P_n) = \{v_1, v_2\} \cup \{v_3, v_4, \cdots\cdots, v_{n+2}\}$ be the vertices of $P_2 + P_n$and the edges of $P_2 + P_n$are $E(P_2 + P_n) = \{v_1 v_2, v_3 v_4, v_4 v_5 \cdots\cdots, v_{n+1} v_{n+2}\} \cup \{v_i v_j\}$, for all $i = 1, 2$, $j = 3, 4 \dots, n + 2$. Thus, the number of edges in $P_2 + P_n$is 3n. Define a labeling function $f: E(P_m + P_n) \to \{1, 2, \cdots, k\}$, such that,
$f(v_i v_{i+1}) = i, i = 1, 2, \dots n + 2$ *where* $v_{n+3} = v_1$. The edge $v_2 v_5$is labeled with an odd multiple of 3. And the remaining $2n - 3$ edges are labeled with distinct prime numbers.

We now show that, the above mentioned labeling is a coprime edge labeling. For the vertex $v_1$ , the edges incident on $v_1$ have the labels $1, n+2$ , and prime numbers, which are relatively prime. Similarly for the vertex $v_2$, the edges incident on $v_2$ have the labels $1, 2$, odd multiples of 3 and prime numbers, which are relatively prime.

Also for the vertices$v_i$ , $i = 3,4, \dots n+2$ except$v_5$, the edges incident on $v_i$will have the labels $i-1, i, and\ p_i's$, which are relatively prime. Finally for the vertex, $v_5$the edges incident on $v_5$will have the labels $4, 5, odd\ multiple\ of\ 3,\ and\ p_i's$ which are relatively prime. Hence the above defined labeling is a coprime edge labeling. To find the minimum coprime edge number, it is enough to find the minimum number of primes used in the above said labeling. Thus the maximum prime numbers used to label the edges of $P_2 + P_n$are$\pi(n+2) + [2n-3]$. That is, $p\tau_E(P_2 + P_n) \leq p_{\pi(n+2)+[2n-3]}$.

**Relatively Prime Index of Graphs**

In this section, we discuss the case in which the minimum coprime edge labeled graph becomes a relatively prime edge labeled graph, by removing some edges in G. This leads to the following definition.

**Definition 5.1** Let G be a coprime edge labeled graph. A relatively prime index of G is defined to be the minimum number of edge removal, resulting in a relatively prime edge labeled graph$G^*$. And it is denoted by, $\varepsilon_r(G)$. In other words,

$\varepsilon_r(G) = \min\{e_i \mid G^* = G - \{e_i\}$ is a relatively prime edge labeled graph$\}$

In the following theorem, we find the relatively prime index number of the complete graph$K_n$and the corona product of $K_n$and$K_1$.

**Theorem 5.2** For a graph $K_n \odot K_1, \varepsilon_r(K_n \odot K_1) = \begin{cases} \frac{n(n-3)}{2} & , if\ 2n+1 \equiv 0(mod\ 3) \\ \frac{n(n-3)}{2} - 1 & , if\ 2n+1 \not\equiv 0(mod\ 3) \end{cases}$

**Proof**

Let $G = K_n \odot K_1$ be the graph with2n vertices and$\frac{n(n+1)}{2}$edges and let $v_1,\ v_2,\ \cdots\cdots, v_n, u_1,\ u_2,\ \cdots\cdots, u_n$ be the vertices of$K_n \odot K_1$.

Case 1: For $2n+1 \equiv 0(mod\ 3)$.

It is enough to prove that, the removal of $\frac{n(n-3)}{2}$edges results in a relatively prime edge labeled graph. Suppose the removal of $\frac{n(n-3)}{2} - 1$ edges in $K_n \odot K_1$results in a relatively prime edge labeled graph. That is, remaining $\frac{n(n+1)}{2} - \frac{n(n-3)}{2} + 1 = 2n+1$ edges of $K_n$ can be labeled from 1 to $2n+1$. Hence by removing $\frac{n(n-3)}{2} - 1$ interior edges of $K_n \odot K_1$, the resultant graph will be of the form $C_n$ with n edges,n pendent vertices $(u_1,\ u_2,\ \cdots\cdots, u_n)$ connecting to the each vertex of $C_n$ and an edge connecting any two non-adjacent vertices of $C_n$ .Each vertex$v_1, v_2, \cdots\cdots, v_n$of $C_n$ is of degree 3. By labeling the edges of$C_n$ with $1, 3, 5, \dots . 2n-1$, each edge incident on the pendant vertices is labeled with $2, 4, 6, \dots, 2n$and an edge connecting any two non-adjacent vertices of $C_n$ is labeled with $2n-1$.As $2n-1 \equiv 0(mod\ 3)$, that is$2n-1 = 3m$the label incident on the vertices of the edge with label $2n-1$ fails to be relatively prime.

Case 2: For$2n-1 \not\equiv 0(mod\ 3)$

It is enough to prove that, the removal of $\frac{(n-3)}{2} - 1$ edges results in a relatively prime edge labeled graph. Suppose the removal of $\frac{(n-3)}{2} - 2$ edges in $K_n \odot K_1$results in a relatively prime edge labeled graph. That is, remaining $\frac{(n+1)}{2} - \frac{(n-3)}{2} + 2 = 2n-2$ edges of $K_n$ can be labeled from $1$ to $2n-2$. Hence by removing $\frac{(n-3)}{2} - 2$ interior edges of $K_n \odot K_1$, the resultant graph will be of the form $C_n$ with n edges, n pendent vertices $(u_1, u_2, \cdots\cdots, u_n)$ connecting to the each vertex of $C_n$ and two edges connecting any two non-adjacent vertices of $C_n$ . Each vertex $v_1, v_2, \cdots\cdots, v_n$of $C_n$ is of degree 3. By labeling the edges of$C_n$ with$1, 3, 5, \dots . 2n-1$ , each edge incident on the pendant vertices is labeled with $2, 4, 6, \dots, 2n$and the edge connecting any two non-adjacent vertices of $C_n$ is labeled with $2n+1, 2n+2$. As $2n+1 \not\equiv 0(mod\ 3)$, the label incident on the vertices of the edge with label $2n+2$ fails to be relatively prime.

□

Illustration 5.3 Illustration of the above theorem for $K_5 \odot K_1$ is given below

## CONCLUSION

The work presented here provides some new results in the theory of minimumcoprime edge labeling of graphs. The main focus of this study is to find the minimumcoprime edge number of complete bipartite graph $K_{2,n}$ and $K_{3,n}$ and also an upperbound for the complete graph $K_n$. Finally, a relatively prime index number of coronaproduct of $K_n$ and $K_1$ is found. In future, relatively prime index of some class ofgraphs can be determined.

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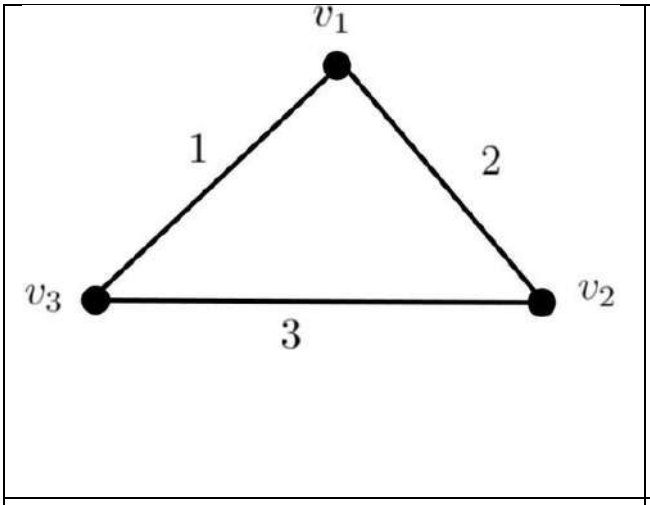


**Figure 1: Relatively prime edge labeling of $C_3$**

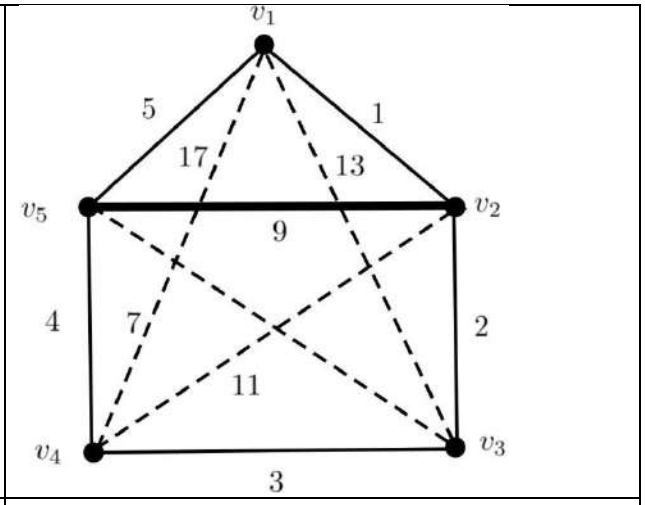


**Figure 2: Coprime edge labeling of $K_5$**

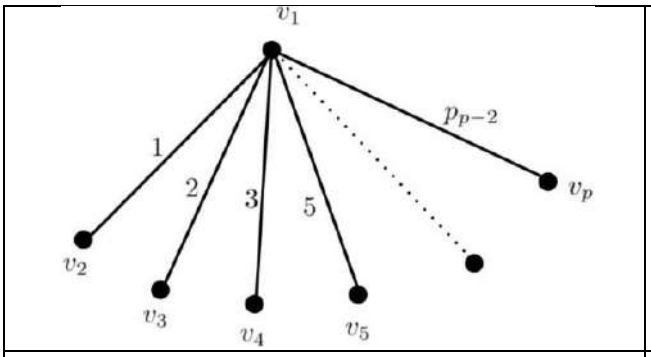


**Figure 3: Coprime edge labeling**

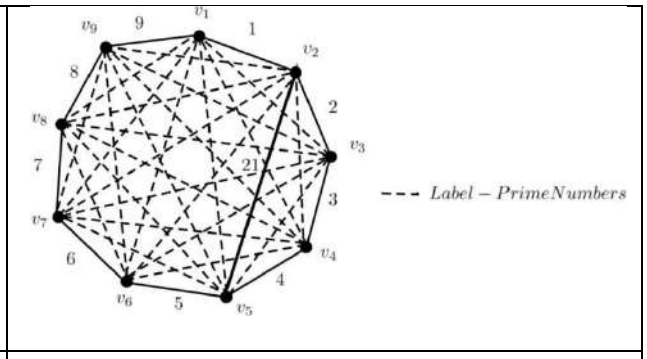


**Figure 4: Coprime edge labeling for $K_9$**

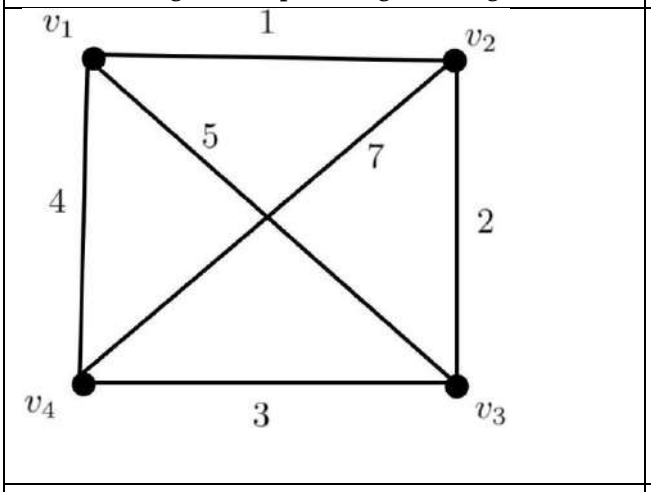


**Figure 5: Minimum Coprime edge labeling of $K_4$**

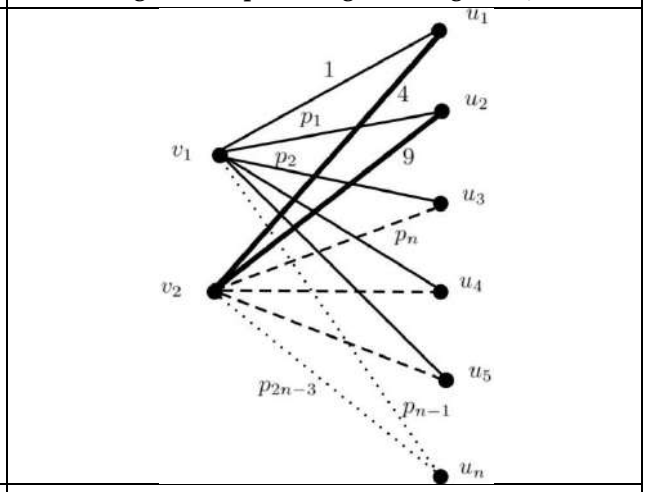


**Figure 6: MinimumCoprime edge labeling of $K_{2,n}$**

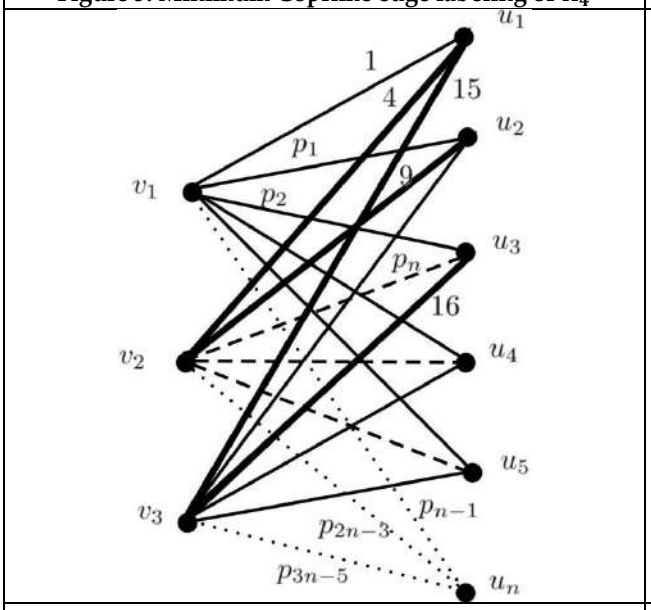


**Figure 7: Minimum Coprime edge labeling of $K_{3,n}$**

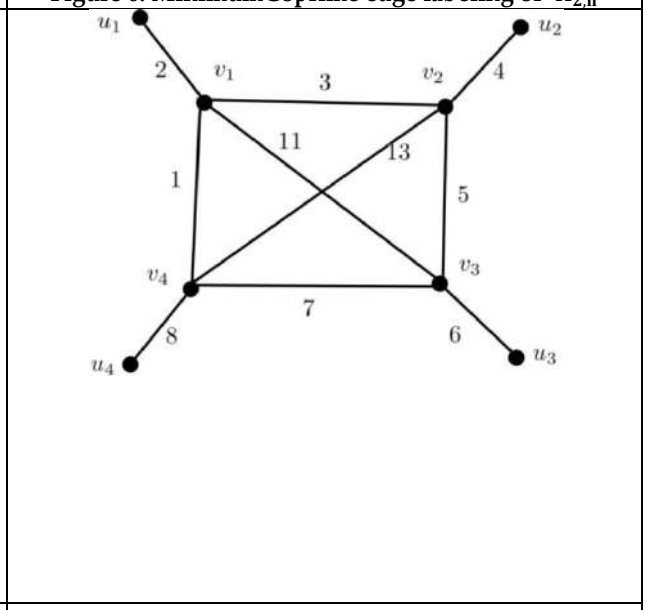


**Figure 8: Minimum Coprime edge labeling of $K_4 \odot K_1$**

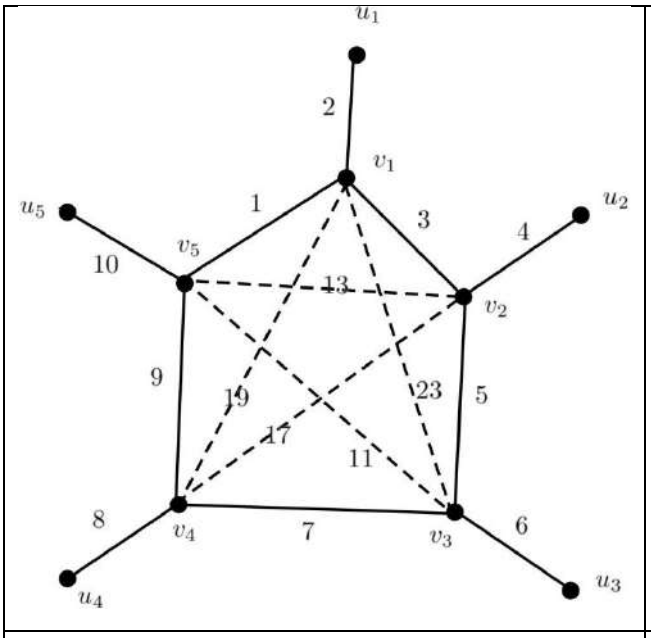


**Figure 9: Minimum Coprime edge labeling of $K_5 \odot K_1$**

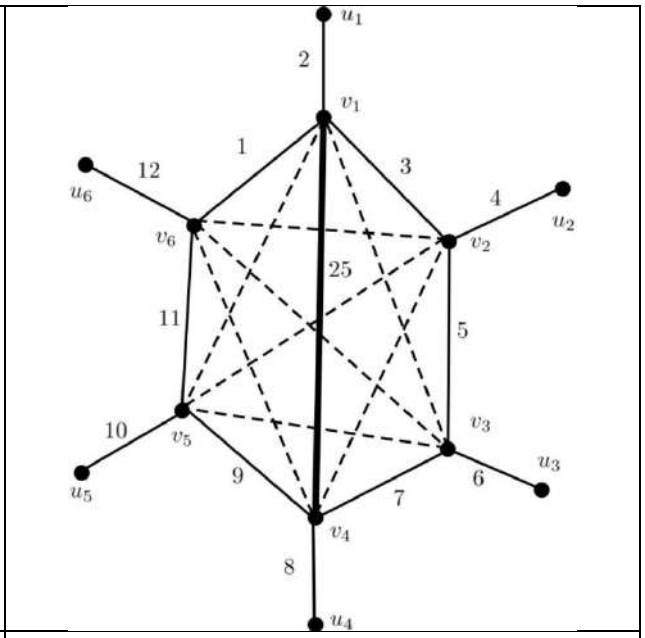


**Figure 10: Minimum Coprime edge labeling of $K_6 \odot K_1$**

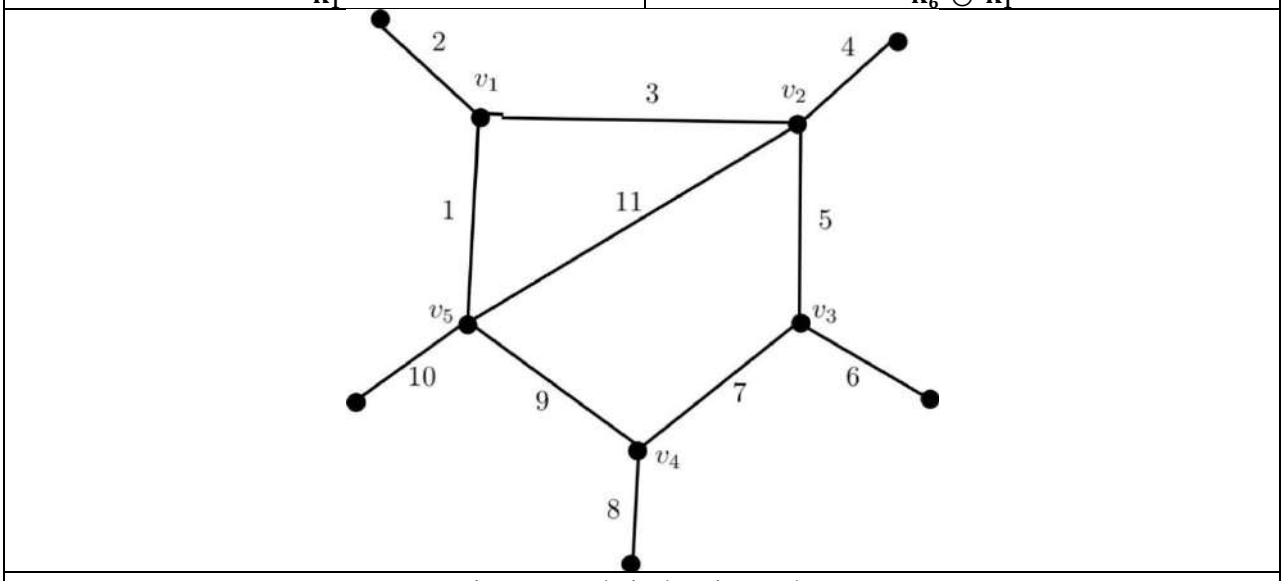


**Figure 11: Relatively Prime Index**

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**RESEARCH ARTICLE**

# A Study on Potted Plants in the Removal of Indoor Air Pollutants

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**ABSTRACT**

This study reviewed quantitative empirical research on the effects of indoor plants on air quality. The information sources were the Web of Knowledge and Pubmed. The eligibility criteria included studies with (1) interventions using any indoor plant (2) comparators included within the same experimental treatment (3) results that included air quality effects objectively measured using any instrument, (4) research using a controlled study design (5) articles published in English. A Total of seventeen research articles reporting quantitative empirical research which were published between 2011 to 2022 were selected. 17 reviewed articles are compiled according to (1) Inferences (2) intervention (3) plant name (4) type of pollutant (5) experimental setup (6) research environment. The effects of indoor plants on air quality were reduced pollutant levels (particularly Volatile organic compounds, formaldehyde, Toluene, and ethyl benzene), followed by smoke particles, Nitrogen dioxide, Carbon dioxide, and Carbon monoxide.This study has shown that indoor plants have a significant potential to reduce air pollution, improve indoor occupant comfort, and overall improve the public health.

**Keywords:** Indoor air quality, Indoor plants, PM10,PM2.5, VOC.

## INTRODUCTION

One of the primary issues in towns and cities, particularly in developing nations is air pollution. Indoor environments were a combination of indoor contaminants which originate inside the building and outside contaminants which are frequently associated with industrial activity and automobile traffic. Outside pollutants can enter through natural and mechanical ventilation systems, infiltrations, or other means. **[1]**. Chemical complexes found in common household cleaning products, fungicides, insecticides, textiles and clothing, paints, couches, and

other pesticides are another form of air pollution. [2]The primary cause of indoor pollution in public spaces like restaurants, cafés, and other closed public areas is cigarettes. When compared to comparable public spaces where smoking is prohibited, the levels of PM2.5 in areas where smoking is permitted are significantly greater.[3]The necessity for air pollution controls has been brought to light by growing concern over the harmful impact of air pollution on health.[4]In the industrialized world, the quality of the indoor environment has become a major health problem since individuals who live in cities usually spend 80–90% of their time indoors. [5]. People may experience ailments like sick building syndrome, which is characterized by headache, eye, nose, and throat irritation, weariness, dizziness, and nausea, if indoor air quality is inadequate. [6]about 1.2 million people died in India in 2017 as a result of indoor and outdoor air pollution (Health Effects Institute 2019). This is mostly due to the fact that energy-saving measures such as sealing up naturally occurring apertures in buildings, utilizing unproven new materials, and inadequate air circulation significantly reduce indoor air quality. The interference is causing a rise in indoor concentrations of many pollutants, including CO, CO2, PM10, PM2.5 and total volatile organic compounds.[7]Studies conducted recently have shown that indoor plants may dramatically lower most forms of urban air pollution. [8], [11]. Through mechanisms like rhizosphere biodegradation (by microorganisms), phytoextraction (plant-liquid extraction), stomatal uptake (plant-gas extraction), phytodegradation (via enzymatic catalysis inside tissues), and phytovolatilization (directly by evaporation from leaves or indirectly by plant transpiration), ornamental plants have the capacity to absorb, distribute and transport organic pollutants. [5]The goal of this study was to compile information in order to give a broad picture of how indoor plants affect the indoor air quality

## MATERIAL AND METHODS

The databases like Science Direct, PubMed, Web of Knowledge, and Google Scholar were searched for any available scientific literature published in the previous ten years (2011–2022). **[9]**The search terms entered alone and in combination included ''Indoor Air Pollution'', Indoor plants'', ''Volatile Organic Compounds'', ''Environmental Exposure'', ''Volatile organic compounds'', "PM10" and "PM2.5". literature was screened using the subsequent search parameters: (1) treatments utilizing any indoor plant; (2) comparators incorporated into the same experimental treatment; (3) outcomes including objectively measurable air quality impacts using any instrument; (4) studies employing any study design; and (5) publications written in English.[**6]**Only Journal publications with quantitative empirical research were used for this study. Thus, works such as "Vegetated Facades as Environmental Control Systems, Filtering Fine Particulate Matter (PM2.5) for Improving Indoor Air Quality and unpublished theses and dissertations were excluded. Real data and analysis are usually used in empirical research, whereas statistical, mathematical, and computational techniques are used in quantitative research. Furthermore, because they must undergo peer review, Journal publications published in the public domain are subject to stricter publication requirements also Compared to laboratory trials, field studies carried out in real ecosystems have more ecological validity.

**Data Collection**

Electronic databases are searched by using the keywords and the paper titles and abstracts are examined based on the previously indicated eligibility requirements. If the article titles and abstracts were not enough to make a decision, the full contents were evaluated. Every study published in each publication was covered in the study. After the articles were examined based on the qualifying criteria, their entire texts were retrieved

**Data Items**

The study includes: author name, year of publication, intervention (number and condition of plants), scientific names of the plants, design study, effect on air quality, and type of pollutants, research environment, ventilation condition, and climatic condition, duration of pollution exposure, period of sampling, measurement replication, number and results

## RESULTS

After the search using the keywords on the Web of Science, Pubmed etc. total 185 research and review papers published were identified and 17 research article involving in an experimental setup (controlled chambers) and are published between 2011 to 2022 were selected.(Table 1). In the 17 research articles selected 37 plant species were tested for their effects on air quality.(Table 2). To avoid misjudgements during data compilation, J. articles containing incomplete or unrecognizable species, non-specified scientific names, or incorrect names were excluded.[6]. The most frequently tested indoor plant species was Epipremnumaureum (Pothos) which appeared 4 times followed by Chlorophytumcomosum (Spider Plant) 3 times Fatsia japonica, Syngoniumpodophyllum, Aloe vera, Areca palm and Ficusbenjamina2 times each and rest of the plant species appeared only 1 times. Table 2 lists the 38plant species used in thisstudy.

Table 3 shows that in the reviewed J. articles Volatile organic compounds was removed by 16 plants followed by Formaldehyde gas 13, Toluene and ethylbenzene 13, smoke particle 5, Nitrogen dioxide 3, Carbon dioxide 2 and Carbon monoxide (CO) by 1 plant. (Table 3). The experiments on plants to remove indoor air pollutants in the reviewed J. articles are done at 23 to 25 $^{o}$C with relative humidity as 50 to 70% in most of the cases. In most of the reviewed articles plants are tested in a controlled environment by making glass air tight chambers of varying size from 26 x 20 x 30.5 cm to 183 cm × 168 cm × 168 cm internal dimensions. Some tests in the reviewed articles are also performed in the natural environment.

## CONCLUSION

According to several studies, potted plants may significantly lower indoor air pollution by eliminating a variety of important pollutants. For these reasons, potted plants are a very portable, affordable, self-regulating, adaptable, flexible, attractive, and sustainable bio filtration system. The majority of the plants under study are capable of eliminating volatile organic chemicals majorly followed by smoke particles, carbon dioxide, nitrogen dioxide,ethylbenzene, formaldehyde gas and carbon monoxide (CO). Additional empirical research and field trials are necessary to determine the precise link between the plants and pollutants, even if the results mentioned above were mostly acquired in labs. Nevertheless, they have significant reference and application value for policymakers and environmental planners. Furthermore, it is advised to do laboratory tests to look into causal linkages, with field experiments following to validate the lab results.This study has shown that indoor plants have a significant potential to reduce air pollution, improve indoor occupant comfort, and overall improve public health.

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**Ethical Approval**
There is no ethical issue for this article.

**Conflicts of Interest**
The author declare that they have no conflicts of interest.

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**Table 1 Summary of reviewed studies of indoor plant effects on air quality.**

| Author | Inferences | Interventions | Plant name | Type of pollutants | Experimental setup | Research environment |
|---|---|---|---|---|---|---|
| Jinhee Lee et.al.2015 **[10]** | Plant can remove 29-35% tobacco pollutants in 60 minutes. | 30 air-filtering plants which can grow at minimum temperature of 10°C and minimum light of 500 LUX in all year are selected . | Spathyphyllurm spp. Samsevieriatrifasciata, Rhapis excels, Fatsia japonica, Cupresscusmacrocarpae 'Gold crest' | smoke particle pollutant | Closed chamber (0.9*0.6*0.6 m volume 0.32 $cm^3$) for almost ground cover plants. | Minimum temperature of 10°C and minimum light of 500 LUX. |
| Fraser Torpy and Michael Zavattaro 2018 **[11]** | Air pollutant reduction with two commonly used indoor species. | For each test, a single plant-module was placed and Three replicate planted modules were used in every trial. | Chlorophytumcomosum (Spider Plant) and Epipremnumaureum (Pothos). | particulate matter (PM), and volatile organic compounds (VOCs) | sealed bench-top test-chamber (0.6 x 0.6 x 0.6 m; 0.216 m3) | 100 mm dia. In-built substrate ventilation fans produced airflows of 4.5 or 9.0 L/s. Temp. Maintained: 23–26°C. light intensity:100 µmol/ $m^2$/s |
| P.J. Irga, F.R. Torpy, M.D. Burchett 2013 **[12]** | With a moderate increase in indoor light intensity, Syngoniumpodophyllum removed significant amounts of $CO_2$ | 8 pots for each treatment. Plants were grown for 133 days before testing. The growing conditions were: temperature: 23.0; relative humidity: 45 | Syngoniumpodophyllum | volatile organic compounds (VOCs) and $CO_2$ | Eight glass test chambers, 26x 20 x 30.5 cm (interior volume 15 L) equipped with a portable Infrared Gas Analyser (IRGA) and $CO_2$ monitor | Fans 50 mm diameter were fitted to maintain air circulation. Temperature in the chambers was maintained at 23°C throughout the |

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| | | | | | | experiments with internal copper coil (i.d. 4 mm) circulating water from a thermostat-regulated water bath. |
| Suya Zhao et.al. 2018 **[13]** | Results show two plants have strong tolerance to formaldehyde in the air and good formaldehyde removal ability. | Uniform seedlings were individually selected and transplanted into 4-L containers filled with 1/5-strength Hoagland nutrient solution. The plants were grown hydroponically for two weeks before experiment | Wild Taraxacummongolicum Hand.-Mazz. and Plantagoasiatica L | formaldehyde | transparent glass container (50 × 30 × 35 cm) filled with 1000 mL of a solution containing varying formaldehyde contents. | The container (pot) was sealed and placed into a plant growth room (with a 14-h light period) with temperatures of 25 °C during the day and 20 °C at night and 50–70% relative humidity. |
| Zhiqiang Wang et. al. 2014 **[14]** | Role of the plant was to introduce and maintain a favourable microbe community that effectively degraded the VOCs. | GoldenPothosplants (one or two), with its status just like how it is placed in homes and offices, were placed in the chamber. | Golden PothosEpipremnumaureum | formaldehyde | Tests were conducted in an air tight 5.1 $m^3$stainless steel chamber with interior dimensions of 1.83 m × 1.68 m × 1.68 m. | Temperature and Relative humidity can be controlled |
| ZhongjunXu et. al. 2011 **[15]** | Spider plant–soil system had the highest formaldehyde removal capacity compared with others. | 3 pots of each plant were planted to the porcelain pots with an inner diameter of 15 cm and a height of 10 cm. and have been cultivated in a light intensity of 240 micro mol/ $m^2$/s (12 h in light) for more than 5 months. | spider plant (Chlorphytumcomosum), aloe (Aloe vera) and golden pothos (Epipremnumaureum) | formaldehyde | cylindrical plexiglass chamber with an inner diameter of 40 cm and a height of 60 cm. | potted plant species placed into each dynamic chamber was subjected to the light intensities of 80, 160, 240 micro mol/ $m^2$ /s (12 hour in light) |

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| Suya Zhao et. al. 2019 **[16]** | Removal rate of plants in the plant-only systems were ordered as Helianthus annuus Linn >Lycopersiconesculentum Miller >Oryzasativa>SansevieriatrifasciataPrain>Bryophyllumpinnatum >Mesembryanthemumcordifolium L. f. | Uniform plant seedlings were transplanted into glass bottles containing 50mL of a nutrient solution. Glass bottles were amended with 40mL of nutrient solution and 10mL of soil microorganism solution. The bottles were wrapped with dark paper and the space between the cap and plant shoots was sealed by a sponge wrapped in aluminum foil. | Helianthus annuus Linn, Lycopersiconesculentum Miller, Oryzasativa, SansevieriatrifasciataPrain, Bryophyllumpinnatum, Mesembryanthemumcordifolium L. f | formaldehyde | Transparent glass container (50x30x35 cm) filled with 1L of solution containing varying formaldehyde concentrations. | The container (pot) was sealed and placed in a plant growth room with a 14 h light period (260–350 micro mol/m$^2$/s) at temperatures of 25 OC during the day and 20 $^{O}$C during the night, and 50–70% relative humidity. |
| BhavyaBhargava At. El. 2020. **[17]** | Areca palm potted plants offer an efficient, cost-effective, self-regulating, sustainable solution for improving indoor air quality | (12-month old), free from any insect-pest attack, potted (30 × 30 × 20 cm) in a standard growing media (5 Kg per pot) containing soil, sand, and well-decomposed Farm Yard Manure (1:1:1), were selected | Areca palm | Total volatile organic compounds (TVOCs), Carbon dioxide (CO2), and Carbon monoxide(CO) | Site I: Floriculture laboratory, 122.1 m$^3$), site II Chemistry laboratory, 256.5 m$^3$), site III canteen, 153.4 m$^3$), and site IV (library, 197.2 m$^3$) | Ambient atmosphere. natural ventilation (ceiling and exhaust fans) without any additional air conditioning systems |
| Hakimeh Teiri et. al. 2018 **[18]** | plant efficiently removed formaldehyde from polluted air by 65-100%, depending on the inlet concentration for a long time exposure | | Chamaedoreaelegans | formaldehyde | test chamber with a volume of 375 liter (84 cm x length × 62 cm width × 72 cm height) | temperature and relativehumidity of the chambers were controlled by digital hygro-thermometers. The light intensity, supposed to be natural indoor |

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| | | | | | | environment light four times a day over the experimental periods using. |
| Todd A. Wetzel & William J. Doucette 2014 **[19]** | Plant leaves to be used as cost effective, real-time indoor air VOC samplers. | Plants were kept under a 400-W metal halide grow lamp with a 10-h photoperiod and were watered as needed to maintain health. | Ficusbenjamina, EpipremnumaureumChlorophytuscomsosum 'vittatum' andSchlumbergera truncate 'harmony'. | Volatile organic compounds | Actual residence room | The residence was equipped with a forced air (HVAC) system and had a total air volume, including the basement, of 600 $m^3$. house thermostat set at 25.6 $^oC$. |
| Curtis Gubb et.al. 2022 **[20]** | Potted plants offer clear potential to improve indoor air quality. | 3 L containers (19 cm wide and 15 cm tall, with 227 $cm^2$ surface area. plants were kept at room temp. (21–22 °C) and light levels (~ 500 lx) | Dracaena fragrans 'Golden Coast', Spathiphyllum wallisii 'Verdi' and Zamioculcasza miifolia | $NO_2$ Nitrogen dioxide. | 150 L (45 X 45 X 75 cm, 0.15 $m^3$) Perspex chamber. 'no' light (0 lx) was achieved by Undertaking exp. at night. | Initial $NO_2$ concentration of 100 ppb (± 15%). Experiments were conducted for a duration of 1 h. |
| Elham F. Moha et.al. 2015**[21]** | Removal rate was found to be more than 67% in the treated chamber. | Storage bottle (250 mL) containing liquid formaldehyde, an air pump, a gas flow meter, and a set of two plastic chambers with an inside volume of 0.512 $m^3$. | sugarcane bagasse waste raw materials with Four well-established potted plants (petunias) | formaldehyde gas | Two plastic chambers with an inside volume of 0.512 $m^3$ with 0.8m each side. HCHO concentration was in the range of 15–20 $mg/cm^3$. | The plants were exposed to HCHO gas during 18 hr. along several time intervals that increased gradually during the experiment progress. The total period of plants exposure was 7 days. |
| MajbritDela Cruz et.al. 2018 **[22]** | All gasoline VOCs were reduced when H. helix was present. total VOC removal was in the range of | Plants were 6 weeks old, grown in 11 cm pots and consisted of 6-7 plantlets. plants were let to | Hedera helix 'Shamrock' | volatile gasoline compounds | Plant was placed in two of four glass chambers (57.5 L) Light | Entire potted plant was exposed to gasoline. The mixing |

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| | 11-32%. | acclimate for 14 days. after the first 15 days the epigeous plant material was abscised and for the last eight days only the pot with soil and the hypogeal part remained. | | | intensity was set to 37 ± 3 micro mol/m²/s and the day length was 12 h. Temperature was controlled at 20.8 ± 0.5 °C. | chamber was supplied with a 200 mL bottle with gasoline, which could diffuse through a 1.7 cm 12 gauge needle in the lid of the vial. The air flow was regulated at 4.3 ± 0.1 L/ min by a pressure regulator. |
| Vanessa Horman net. al. 2017 **[23]** | Rapid decline in the toluene as well as in the 2-ethylhexanol concentrations was observed when plants were present in the chamber. | Three plants of either D. maculata or S. wallisii or three pots without plants filled with unused potting soil only were tested per Biobox under continuous light. All experiments were replicated four times. | Dieffenbachia maculata and Spathiphyllum wallisii | Toluene and 2-ethylhexanol | Two individual gas-tight chambers 80 × 60 × 50 cm with a total volume of 240 L consists of two parts: a metal base 15 × 60 × 50 cm and a Plexiglas hood (65 × 60 × 50 cm) on top. | $CO_2$ = 500 ppm, RH = 70%, temperature = 22 °C, light =180 ± 10 μmol/m²/s |
| Kwang Jin Kim et. al. 2018 **[24]** | Root zone is a major contributor to the removal of formaldehyde. | Two-year-old plants were transplanted to 19 and 15 cm dia. pots. plants were kept in indoor env. for more than 1 month at 23°C ± 2 °C, 40% ± 5% relative humidity, and a light intensity of 20 ± 2 mol/m²/s. | Fatsia japonica Decne. andFicusbenjamina. | formaldehyde | Air tight chamber of 1.0 m³ (90 cm X 90 cm X 123 cm) air was circulated (6 L/min) and released at the bottom of the chamber. | |
| Wararat Sriprapat et. al. 2014 | Highest toluene removal was found in S. trifasciata, while ethylbenzene removal | Twelve plant species with leaf area of 0.013 m² was chosen for the | Aloe vera, Sansevieriamasoniana, Sansevieriatrifas | toluene and ethylbenzene | Glass chambers with volume 15.6 L were | Toluene or ethyl benzene was injected to generate |

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| **[25]** | from air was with C. comosum. | experiment cultures of plants were maintained in plastic pots (0.1X 0.1 $m^2$ ) contain 200 g of soil and coco coir 1:1 as growth media. the pot was covered by aluminum foil to avoid other factors such as soil and pot absorption. | ciata, Sansevieriahyacinthoides, Sansevieriaehrenbergii, Kalanchoe, blossfeldiana, Dracaena deremensis Codiaeumvariegatum, Chlorophytumcomosum, Dracaena sanderiana Cordylinefruticosa Aglaonemacommutatum. | volatile compounds | used for plant fumigation. Three replicate chambers were used in each treatment. | the concentration of 20 ppm or 12 micro mol inside the chamber. Plant was placed into each chamber. at room temp. (32°C) and a pressure of 760 mmHg with 12 h of light and dark cycles |

**Table 2 Most frequently studied plant families and species**

| **Plant Family** | **Frequency** |
|---|---|
| Epipremnumaureum (Pothos) | 4 |
| Chlorophytumcomosum (Spider Plant) | 3 |
| Fatsia japonica | 2 |
| Syngoniumpodophyllum | 2 |
| Aloe vera | 2 |
| Areca palm | 2 |
| Ficusbenjamina | 2 |
| Spathyphyllurmspp | 1 |
| Samsevieriatrifasciata | 1 |
| Rhapis excels | 1 |
| Cupresscusmacrocarpae | 1 |
| Wild Taraxacummongolicum Hand | 1 |
| Plantagoasiatica L | 1 |
| Helianthus annuus Linn | 1 |
| Lycopersiconesculentum Miller | 1 |
| Oryzasativa | 1 |
| SansevieriatrifasciataPrain | 1 |
| Bryophyllumpinnatum | 1 |
| Mesembryanthemumcordifolium L. f | 1 |
| *Chamaedoreaelegans* | 1 |
| Christmas cactus (Schlumbergera truncate 'harmony') | 1 |
| Dracaena fragrans 'Golden Coast' | 1 |
| Spathiphyllumwallisii 'Verdi' | 1 |
| Zamioculcaszamiifolia | 1 |
| sugarcane bagasse | 1 |
| Hedera helix 'Shamrock' | 1 |

| | |
|---|---|
| Dieffenbachia maculata | 1 |
| Spathiphyllumwallisii | 1 |
| Sansevieriamasoniana | 1 |
| Sansevieriatrifasciata | 1 |
| Sansevieriahyacinthoides | 1 |
| Sansevieriaehrenbergii | 1 |
| Kalanchoe, blossfeldiana | 1 |
| Dracaena deremensis 'Lemon lime' | 1 |
| Codiaeumvariegatum, | 1 |
| Cordylinefruticosa | 1 |
| Aglaonemacommutatum | 1 |
| Dracaena sanderiana | 1 |

**Table 3 Type of pollutant removed by the plant species**

| **Plant name** | **Type of pollutant** |
|---|---|
| Epipremnumaureum (Pothos) | formaldehyde |
| Chlorophytumcomosum (Spider Plant) | formaldehyde |
| Fatsia japonica | Formaldehyde and smoke particle pollutant |
| Syngoniumpodophyllum | volatile organic compounds (VOCs) and CO2 |
| Aloe vera | toluene and ethylbenzene volatile compounds |
| Areca palm | Total volatile organic compounds (TVOCs), Carbon dioxide (CO2), and Carbon monoxide (CO) |
| Ficusbenjamina | Volatile organic compounds |
| Spathyphyllurmspp | smoke particle pollutant |
| Samsevieriatrifasciata | smoke particle pollutant |
| Rhapis excels | smoke particle pollutant |
| Cupresscusmacrocarpae | smoke particle pollutant |
| Wild Taraxacummongolicum Hand | formaldehyde |
| Plantagoasiatica L | formaldehyde |
| Helianthus annuus Linn | formaldehyde |
| Lycopersiconesculentum Miller | formaldehyde |
| Oryzasativa | formaldehyde |
| SansevieriatrifasciataPrain | formaldehyde |
| Bryophyllumpinnatum | formaldehyde |
| Mesembryanthemumcordifolium L. f | formaldehyde |
| *Chamaedoreaelegans* | formaldehyde |
| Christmas cactus (Schlumbergera truncate 'harmony') | Volatile organic compounds |
| Dracaena fragrans 'Golden Coast' | $NO_2$ Nitrogen dioxide. |
| Spathiphyllumwallisii 'Verdi' | $NO_2$ Nitrogen dioxide. |
| Zamioculcaszamiifolia | $NO_2$ Nitrogen dioxide. |
| sugarcane bagasse | Formaldehyde gas |
| Hedera helix 'Shamrock' | volatile gasoline compounds |
| Dieffenbachia maculata | Toluene and 2- ethylhexanol |
| Spathiphyllumwallisii | Toluene and 2- ethylhexanol |
| Sansevieriamasoniana | toluene and ethylbenzene volatile compounds |

| | |
|---|---|
| Sansevieriatrifasciata | toluene and ethylbenzene volatile compounds |
| Sansevieriahyacinthoides | toluene and ethylbenzene volatile compounds |
| Sansevieriaehrenbergii | toluene and ethylbenzene volatile compounds |
| Kalanchoe, blossfeldiana | toluene and ethylbenzene volatile compounds |
| Dracaena deremensis 'Lemon lime' | toluene and ethylbenzene volatile compounds |
| Codiaeumvariegatum, | toluene and ethylbenzene volatile compounds |
| Cordylinefruticosa | toluene and ethylbenzene volatile compounds |
| Aglaonemacommutatum | toluene and ethylbenzene volatile compounds |
| Dracaena sanderiana | toluene and ethylbenzene volatile compounds |

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**RESEARCH ARTICLE**

# A DFT Study on Natural Sensitizer with Donor - π - Acceptor Architecture based on Luteolin for Applications in Dye - Sensitized Solar Cells

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## ABSTRACT

In the present study, we employed Density Functional Theory (DFT) and Time-Dependent Density Functional Theory (TD-DFT) methodologies to systematically analyse the electronic, optical, and photovoltaic properties of six novel D-π-A type organic dyes denoted as Lu1–Lu6, specifically designed for use in Dye-Sensitized Solar Cells (DSSCs). The optimization andDFT investigations were conducted using the B3LYP density functional in conjunction with the 6-311++G (d, p) basis set for geometry optimization and electronic structure studies, respectively. Parameters such as the free energy change of electron injection ($\Delta G^{inject}$), maximum absorption wavelength($\lambda_{max}$), oscillator strengths (f), light harvesting efficiency (LHE), and dye regeneration ($\Delta G^{reg}$) were computed, along with the vertical electron excitation energy, in both gas and solvent phases. Furthermore, the intermolecular charge transfer of the designed dyes (Lu1–Lu6) was probed through the analysis of frontier molecular orbitals. Our findings indicate that all investigated dyes exhibit favourable characteristics, positioning them as promising candidates for efficient DSSC sensitizers.

**Keywords:** DFT, DSSC,HOMO-LUMO, LHE,natural dyes

# INTRODUCTION

Researchers are highlighting the significance of environmentally friendly dye-sensitized solar cells (DSSC), also known as Gratzel cells, in response to the emergence of clean and cost-effective renewable energy [1]. In the realm of solar energy conversion, DSSCs offer a more economical alternative compared to traditional semiconductor (SC) photovoltaic devices [2-3]. A typical DSSC device consists of a dye sensitizer, $I^-/I^{3-}$ electrolyte, counter electrode, and $TiO_2$ semiconductor [4]. The dye sensitizer is a critical component in DSSCs, absorbing light and generating photo-excited electrons, and a promising candidate meeting these criteria in the metal-free D-π-A organic sensitizer [5].The D-π-A organic dye is composed of three parts: a π-spacer, an electron acceptor group (A), and an electron donor group (D) [6-7]. Electron injection into the semiconductor is facilitated in D-π-A dyes by intramolecular charge transfer (ICT) through the π-spacer from the sensitizer's donor group to its acceptor moiety [8-10]. However, organic dyes face challenges such as a weak absorption coefficient and a narrow absorption band in the ultraviolet and visible regions [4,11]. To address this, modifying the donor, π-bridge, and acceptor moieties is a straightforward approach to enhance the dye sensitizer's performance [12].The current focus is on designing dyes with improved efficiency to boost power conversion efficiency. Luteolin, a flavonoid polyphenol widely distributed in various plant families, is examined for its potential. As part of the flavone group, luteolin has a C6-C3-C6 structure with two benzene rings (A, B), an oxygen-containing (C) ring, and a 2–3 carbon double bond [46]. The study systematically explores how donor, acceptor, and π-spacer groups influence the photophysical characteristics of designed dyes to achieve enhanced efficiency. Six new organic dyes, named Lu1-Lu6 for DSSCs, along with luteolin as a π-conjugated system, were developed based on the molecular structure of D-π-A, using diphenylamine (DPA) and triphenylamine (TPA) as the electron donor (D) and $NO_2$ and CN as the electron acceptor (A), as illustrated in Figure 1. The structures of luteolin and the newly designed dyesare depicted inFigure 2.

**Computational Methods**

All computations were carried out using Becke's three-parameter hybrid functional of Becke, Lee, Yang, and Parr (B3LYP) [13], employing the 6-311++G (d, p) basis set in both gas and solvent phases. The investigation encompassed frontier molecular orbitals,excited state energies, optical absorption spectra, electronic injection parameters, and oscillator strength for the designed dyes [14]. Solvent effects were considered for two solvents, Dichloromethane (DCM) and dimethylformamide (DMF), utilizing the polarizable continuum model (PCM) [15]. Gaussian 09 software was employed for all calculations, and Gauss View Version 5.0 was utilized to visualize electron density models at various energy levels [16].

# RESULTS AND DISCUSSION

**Geometry Optimization**

The geometrical parameters of the designed sensitizers were calculated using density functional theory (DFT) at the B3LYP/6-311++G (d, p) level of theory. The optimized geometry structures are shown in Figure 3. The bond lengths between the electron-donor moiety and the π-spacer (d1) and between the electron acceptor and the conjugated π-spacer (d2) are in the range of 1.443-1.503 Å and 1.469-1.496 Å, respectively, for both the gas and solvent phases. The dihedral angles (ϕ1) between the donor and the π-spacer and (ϕ2) between the π-spacer and acceptor are similar for all the dyes in both the gas and solvent phases. The values of ϕ1 and ϕ2 show that the region between the donor and π-spacer unit and the π-spacer and acceptor unit are planar, respectively. These results suggest that the designed sensitizers have coplanar structures, which is favourable for intramolecular charge transfer (ICT) and DSSC performance. Specifically, the bond length between the electron-donor moiety and the π-spacer (d1) is in the range of 1.443 Å to 1.503 Å for the gas and solvent phases, respectively. The bond length between the electron acceptor and the conjugated π-spacer (d2) is in the range of 1.469 Å in gas phase and 1.469 Å to 1.496 Å for solvent phases, respectively. The dihedral angle (ϕ1) between the donor and the π-spacer is similar for all the dyes. Also, the values calculated for dihedral angle show that the region between the donor and π-spacer unit is planar. The dihedral angles (ϕ2) between π-spacer and acceptor are similar for all the dyes in gas phase and in solvent (ϕ2) the values of

Lu2, Lu4 and Lu6 are around -38.858° which can be ascribed to the steric repulsion between hydrogen atoms that prevent undesirable aggregation of dye on the semiconductor surface. In summary, the values of the dihedral angles presented in Table 1 show that the designed sensitizer Lu1-Lu6 has the most coplanar structure. However, there seems to be no significant differences in the bond length across the optimized phases suggesting that the compound under study possesses insightful properties for DSSC design.

**Molecular electrostatic potential (MEP) surface**

The MEP mapped the surface of designed dyes was computed using DFT/B3LYP at 6-311++G (d, p) basis set and the MEP surface is plotted in Figure. 4 The molecular electrostatic potential (MEP) is useful in determining electrophilic attack sites, nucleophilic reactions, and hydrogen-bonding interactions [50].Colours with increasing electrostatic potential arered>orange>yellow>green>blue, respectively [50].Potentially reactive regions are shown by the colours red for electrophilic reactivity and blue for nucleophilic reactivity [51].The appearance of negative electrostatic potentials(red) around the oxygen in the πspacer and nitrogen atoms of the anchoring acceptor group evidences its negativepartial charge. These sites are potentially relevant for electrophilic attack of the electrolyte,evidenced its negative partial charge. These sites were potentially relevant for electrophilicattacks of the electrolyte.The positive potential site that is exposed to nucleophilic attack is shown by the blue hydrogen bonded to C atomsin the overall ring.

**Frontier molecular orbitals**

The investigation into the dyes' frontier molecular orbitals (FMO) was conducted at the B3LYP/6-311++G (d, p) level of theory to assess electron transfer properties during light excitation[13].Analysingthe highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) provided insights into intramolecular charge transfer behaviour in DSSCs devices [17-18], with the corresponding orbitals depicted in Figure5. The HOMO of the dyes primarily exhibits electron density delocalized on the electron-donor part, while the LUMO is primarily localized on the electron-deficient part with delocalization onto the π-spacer. Both HOMO and LUMO orbitals include contributions from the π-spacer for all designed sensitizers.Efficient electron transfer is facilitated by substantial HOMO-LUMO overlap at the π-spacer, indicating intramolecular charge transfer from the electron-donor (D) to the electron acceptor (A) through the π-spacer. Energy levels of HOMO and LUMO, along with their corresponding energy gaps ($E_{gap}$), are presented in Figure 6 and Table 2. The fundamental principle of DSSCs involves producing an oxidized state of the dye by injecting electrons into $TiO_2$'s conduction band (CB) after dye excitation by incident light [19]. For effective electron injection, the dye's LUMO must be higher than the conduction band of $TiO_2$ [20], and for efficient dye regeneration, the HOMO should be below the redox potential energy of the electrolyte [21-24].All designed sensitizers (Lu1-Lu6) exhibit LUMO energies between -2.9361 eV to -2.3362 eV, significantly higher than $TiO_2$'s conduction band (-4.0 eV), while HOMO energies range from -6.00 eV to -5.1138 eV, lower than the redox potential of the electrolyte (-4.80 eV). The HOMO energy level of designed dyes is notably up-shifted compared to the reference dye Luteolin, whereas the LUMO energy level is slightly down-shifted. The incorporation of electron-donating moieties (diphenylamine and triphenylamine) results in higher HOMO energies, while electron-withdrawing substituents decrease the LUMO energy. Dyes with $NO_2$ groups (Lu2, Lu4, and Lu6) exhibit the lowest LUMO energy levels compared to cyano-substituted dyes (Lu1, Lu3, and Lu5). The substitution of auxiliary donor ($CH_3$) has no significant effect on HOMO levels.The energy level diagram (Fig. 4) suggest that all designed dyes have sufficient driving force to be used as sensitisers in DSSCs. A low energy gap ($E_{gap}$) enhances intramolecular charge transfer (ICT) and results in an apparent absorption band in the electronic spectra. Table 2 summarizes the respective energy gaps, ranging between 2.2890 eV to 3.3734 eV. Compared to the reference dye Luteolin, the designed dyes generally have lower ($E_{gap}$) values. Notably, Lu4 has the narrowest band gap, indicating enhanced power conversion efficiency in DSSCs due to increased photon absorption at longer wavelengths.

**Electronic absorption spectra**

The electronic absorption spectra of dyes Lu1-Lu6 were simulated using the TD-DFT/B3LYP functional and 6-311++G (d, p) basis set, as depicted in Figure 7. A comprehensive summary of the photophysical characteristics, encompassing computed absorption wavelength, oscillator strength, vertical excitation energies, and light harvesting

efficiency for the examined dyes are presented in Table 3. Notably, the absorption spectrum of the newly designed sensitizers Lu1-Lu6 exhibits a remarkable red-shift both in the gas and solvent phases compared to the reference dye Lu. The introduction of a polar solvent results in an increased absorption wavelength compared to the gas phase, contributing to an enhanced light-harvesting process.Lu4, with its strong and consistent absorption characteristics across solvents, emerges as a promising candidate for further exploration.The shift towards longer wavelengths in the absorption spectrum of all designed dyes (Lu1–Lu6) signifies their capacity to absorb more light at extended wavelengths, thereby augmenting the efficiency of solar cells.

**Light Harvesting Efficiency (LHE)**

The LHE [25] of investigated dyes is expressed by equation (1)

$LHE = 1 - 10^{-f}$ [25]-------- ---------- (1)

In the study, the symbol (f) denotes the oscillator strength of the dye associated with its maximum absorption wavelength, $\lambda_{max}$ [26]. The dye's responsiveness to light is quantified through its light harvesting efficiency (LHE) [27]. To enhance the photocurrent response in dye-sensitized solar cells (DSSCs), it is desirable for LHE values to be maximized [26]. Table 3 outlines the oscillator strength (f) and LHE for both luteolin and the developed dyes. A higher oscillator strength (f) indicates an improved light absorbance capability of the sensitizer, leading to an enhancement in light harvesting efficiency (LHE). In comparison to luteolin, all the newly formulated dyes exhibit higher (f) values and elevated light harvesting efficiency. This implies that the designed dyes have a greater potential to convert a larger amount of incident light into electricity, showcasing their efficacy in DSSCs.

**Free energy change of electron injection and oxidation potential**

Through the computation of Gibbs energy changes for electron injection ($\Delta G^{inject}$), one can gauge the energy driving force for the injection of electrons from the excited state into the conduction band of $TiO_2$ [28]. The free energy change associated with the electron injection process can be approximated using the equation [29]:

$\Delta G^{inject} = E_{ox}^{dye^*} - E_{CB}^{TiO_2}$---------- (2)

Here$E_{CB}^{TiO_2}$ is −4.00 eV[29]. The excited state oxidation potential of the dye ($E_{ox}^{dye^*}$)is estimated using the equation [30].

$E_{ox}^{dye^*} = E_{ox}^{dye} - \lambda_{max}^{ICT}$ ---------- (3)

In this equation, $E_{ox}^{dye}$represents the oxidation potential energy of the dye in the ground state ($E_{ox}^{dye} = -E_{HOMO}$) and $\lambda_{max}^{ICT}$ is the vertical transition energy corresponding to $\lambda_{max}$[2].Through these calculations, we determined the electron injection free energy change $\Delta G^{inject}$as well as the ground state ($E_{ox}^{dye}$).The outcomes are presented in Table 4a and 4b for gas and solvent phases. It is noteworthy that all designed dyes exhibit a negative$\Delta G^{inject}$indicating that the excited states of the dyes are positioned above the $TiO_2$ conduction band edge, and the process of electron injection from the dye into the semiconductor occurs spontaneously.

**Dye degeneration**

In dye-sensitized solar cells (DSSCs), the regeneration of the dye($\Delta G^{reg}$)stands as a pivotal stage, ensuring the continual functionality of the solar cell [31] that can beexpressed by the equation (4).

$\Delta G^{reg} = E_{ox}^{dye} - E_{I^-/I_3^-}$---------- (4)

Here$E_{I^-/I_3^-}$is the redox potentialenergy of the iodide/triiodideredox electrolyte(-4.8eV) [31].

A lower $\Delta G^{reg}$ signifies a more favourable condition for the regeneration process [32].Table 4a and 4b provides a summary of the calculated $\Delta G^{reg}$ values for the designed dye in gas and solvent phases. This implies that these dyes potentially possess the capability to enhance power conversion efficiency.

**Open circuit Voltage**

The photovoltaic performance of the DSSC is also influenced by the open-circuit voltage ($V_{OC}$) [13], which can be approximated using the equation (5):

$$V_{OC} = E_{LUMO} - E_{CB} \quad (5)$$

A higher LUMO energy level elevates the $V_{OC}$ value, contributing to an overall improvement in the efficiency of DSSC devices [8]. The calculated open-circuit voltage values for the designed dyes are outlined in Table 5. Notably, among the investigated dyes, the open-circuit voltage has been heightened for the cyano-substituted dyes Lu1, Lu3, and Lu5.Lu2 has lower $V_{OC}$ values compared to other sensitizers, especially in the gas phase and DMF.Lu4 exhibits lower $V_{OC}$values, particularly in DMF and DCM.Lu6 shows varying $V_{OC}$values in different phases, with a notable decrease in DMF. The choice of solvent and sensitizer structure can influence the open-circuit voltage, as seen in the variation of $V_{OC}$ values in different phases.

**Electron coupling Constant**

The estimation of electron coupling constant ($V_{RP}$) for a photo-induced charge transfer utilises the extended generalized Mulliken-Hush (GMH) formalism[34].

$$V_{RP} = \frac{\Delta E_{RP}}{2} \quad (6)$$

According to Koopmans approximation the injection driving force can be expressed as [35]

$$\Delta E_{RP} = [E^{dye}_{LUMO} + 2E^{dye}_{HOMO}] - [E^{dye}_{LUMO} + E^{dye}_{HOMO} + E^{TiO_2}_{CB}] \quad (7)$$

Where $E^{TiO_2}_{CB}$ =(−4.0 eV) and $-E^{dye}_{HOMO} = E^{dye}_{OX}$ [36]

Thus equation (7) can be modified as

$$\Delta E_{RP} = [E^{dye}_{HOMO} + E^{TiO_2}_{CB}] = -[E^{dye}_{OX} + E^{TiO_2}_{CB}] \quad (8)$$

A higher rate constant is obtained with a larger $V_{RP}$, indicating a more effective sensitizer. Strong electronic coupling between the investigated dyes and the $TiO_2$ surface leads to increased electron injection efficiency as shown in Table 5.

**Chemical Reactivity Parameters**

The following chemical reactivity parameters were calculated for evaluatingthe photoelectrical properties of dyes:ionization potential (IP), electron affinity (EA), chemical potential (μ) chemical hardness (η), chemical softness (S), electrophilicity index (ω), electron donatingpower ($\omega^-$), and electron accepting power ($\omega^+$) [38,40] by utilizing the following equations:

$$\mu = \frac{IP+EA}{2} \quad (9)$$

$$\eta = \frac{IP-EA}{2} \quad (10)$$

$$S = \frac{1}{\eta} \quad (11)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (12)$$

$$\omega^+ = \frac{(IP+3EA)^2}{16(IP-EA)} \quad (13)$$

$$\omega^{-} = \frac{(3IP+EA)^2}{16(IP-EA)} \quad \text{----------(14)}$$

An increased electron affinity (EA) can enhance the electron-accepting capability, while a reduced ionization potential (IP) is advantageous for enhancing hole-creating ability [39]. According to molecular orbital theory, the first ionization energy IP = $-E_{HOMO}$ and electron affinityEA = $-E_{LUMO}$ [37]. The computed ionization potentials and electron affinities of the studied dyes are outlined in Table 6. In contrast to the reference dye Lu, the designed dyes display lower ionization potentials (IP), implying that structural modifications may render the dyes more predisposed to losing electrons, thereby improving their photoelectrical properties. Moreover, in comparison to the reference dye Luteolin, the designed dyes exhibit higher electron affinities, indicating a greater tendency for electron absorption.Lu1 has the lowest IP, making it easier to lose an electron compared to other dyes.Lu4 has the highest EA, indicating a strong tendency to accept an electron. Chemical hardness, serving as an indicator of the dyes' resistance to the intramolecular charge transfer (ICT) process, implies that lower chemical hardness advantageous for increased charge transfer and separation [41]. Table 6 indicates that the η values of the designed dyes are lower than those of the reference dye Lu. Notably, Lu4 dyes demonstrate the lowest chemical hardness and highest softness among the studied dyes, suggesting a lower resistance to intramolecular charge transfer. The value of ω can be utilized to calculate the stabilization energies of molecular structures, with higher $\omega^{+}$ values preferred as they indicate the highest electron-accepting capacity [42-44]. Consequently, among the studied dyes, Lu4 dye exhibits the highest electron-accepting power, electron-donating power (ω-), and stabilization energy. Considering these parameters, Lu4 dye possesses the highest electron-accepting capacity, lowest chemical hardness, highest stabilization energy, and, consequently, the highest power conversion efficiency.

## CONCLUSION

We found that the formulated metal-free organic dyes meet essential criteria, encompassing a high molar extinction coefficient, wide absorption spectra, favourable orbital distribution, and satisfactory energy level alignment. The absorption spectra of the crafted organic sensitizer show a red shift compared to the Luteolin dye. All the created dyes have demonstrated a substantial driving force for electron injection, potentially resulting in a higher short-circuit current density. We propose that the devised metal-free organic dyes could represent the most promising choices for enhancing the power conversion efficiency of DSSCs.

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**Table 1 - The Optimized bond lengths and dihedral angles of the dyes at the at B3LYP/6-311++G (d,p) level of theory.**

| Dye | GAS PHASE | | | | DMF | | | | DCM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $d_1$ | $d_2$ | $\phi_1$ | $\phi_2$ | $d_1$ | $d_2$ | $\phi_1$ | $\phi_2$ | $d_1$ | $d_2$ | $\phi_1$ | $\phi_2$ |
| Lu1 | 1.42 | 1.469 | -0.547 | -1.184 | 1.503 | 1.469 | -0.547 | 0 | 1.42 | 1.469 | -0.547 | 0 |
| Lu2 | 1.42 | 1.496 | -0.547 | -1.184 | 1.42 | 1.496 | -0.547 | -38.86 | 1.42 | 1.496 | -0.547 | -38.858 |
| Lu3 | 1.42 | 1.469 | -0.547 | -1.184 | 1.42 | 1.469 | -0.633 | 0 | 1.42 | 1.469 | -0.633 | 0 |
| Lu4 | 1.42 | 1.496 | -0.547 | -1.184 | 1.42 | 1.496 | -0.547 | -38.85 | 1.42 | 1.496 | -0.547 | -38.854 |
| Lu5 | 1.42 | 1.469 | -0.547 | -1.184 | 1.42 | 1.469 | -0.547 | 0 | 1.42 | 1.469 | -0.547 | 0 |
| Lu6 | 1.42 | 1.496 | -0.547 | -1.226 | 1.42 | 1.496 | -0.547 | -38.85 | 1.42 | 1.496 | -0.547 | -38.854 |

**Table 2- The $E_{HOMO}$, $E_{LUMO}$ and energy gap ($E_{gap}$) of dyes in eV at B3LYP/6-311++G (d,p) level of theory**

| DYE | GASPHASE | | | DMF | | | DCM | | |
|---|---|---|---|---|---|---|---|---|---|
| | HOMO | LUMO | $E_{gap}$ | HOMO | LUMO | $E_{gap}$ | HOMO | LUMO | $E_{gap}$ |
| **Lu** | -6.4341 | -2.1723 | 4.2618 | -6.4477 | -2.2767 | 4.1709 | -6.4442 | -2.2591 | 4.1851 |
| **Lu1** | -5.1138 | -2.3362 | 2.7777 | -5.0785 | -2.3731 | 2.7053 | -5.8314 | -2.4580 | 3.3734 |
| **Lu2** | -6.0045 | -2.7766 | 3.2278 | -5.8836 | -3.0030 | 2.8806 | -5.8855 | -2.9736 | 2.9119 |
| **Lu3** | -5.4104 | -2.3946 | 3.0158 | -5.4657 | -2.3981 | 3.0675 | -5.4415 | -2.3957 | 3.0457 |
| **Lu4** | -5.3566 | -2.9361 | 2.4204 | -5.4001 | -3.1013 | 2.2988 | -5.3761 | -3.0871 | 2.2890 |
| **Lu5** | -5.3519 | -2.3823 | 2.9696 | -5.4281 | -2.3967 | 3.0313 | -5.4009 | -2.3935 | 3.0074 |
| **Lu6** | -5.5582 | -2.7614 | 2.7968 | -5.5155 | -3.0090 | 2.5064 | -5.5059 | -2.9793 | 2.5265 |

**Table 3- Absorption maxima ($\lambda_{max}$), Oscillator Strength (f), Light Harvesting Efficiency (LHE), average Light Harvesting Efficiency ($LHE_{avg}$)of dyes at B3LYP/6-311++G (d, p) level of theory**

| DYE | GAS PHASE | | | | DMF | | | | DCM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$ | f | LHE | $LHE_{avg}$ | $\lambda_{max}$ | f | LHE | $LHE_{avg}$ | $\lambda_{max}$ | f | LHE | $LHE_{avg}$ |
| Lu | 323.14<br>264.74 | 0.2848<br>0.0139 | 0.4809<br>0.0314 | 0.2561 | 347.62<br>315.17 | 0.0979<br>0.0556 | 0.2018<br>0.1201 | 0.1609 | 350.00<br>313.97 | 0.0506<br>0.0593 | 0.1099<br>0.1276 | 0.1187 |
| Lu1 | 400.51<br>391.96 | 0.1049<br>0.3565 | 0.2145<br>0.5599 | 0.3872 | 408.53<br>393.89 | 0.1048<br>0.5038 | 0.2144<br>0.6865 | 0.4504 | 417.12<br>359.82 | 0.0132<br>0.7195 | 00299<br>0.8092 | 0.4195 |
| Lu2 | 365.98<br>344.81 | 0.0466<br>0.2103 | 0.1017<br>0.4771 | 0.2894 | 406.26<br>355.91 | 0.0269<br>0.5134 | 0.0600<br>0.6933 | 0.3760 | 400.85<br>355.90 | 0.0285<br>0.3561 | 0.0635<br>0.5595 | 0.3115 |
| Lu3 | 400.93<br>368.27 | 0.0174<br>0.8101 | 0.0392<br>0.8451 | 0.4421 | 462.61<br>369.89 | 0.0185<br>1.1125 | 0.0417<br>0.9228 | 0.4822 | 466.16<br>370.17 | 0.0165<br>1.1509 | 0.0372<br>0.9293 | 0.4832 |
| Lu4 | 379.12<br>366.43 | 0.1658<br>0.3939 | 0.3173<br>0.5962 | 0.4567 | 479.01<br>375.79 | 0.0145<br>0.6387 | 0.0328<br>0.7702 | 0.4015 | 482.77<br>370.40 | 0.0135<br>0.5418 | 0.0306<br>0.7127 | 0.3716 |
| Lu5 | 407.05<br>371.03 | 0.0157<br>0.7834 | 0.0355<br>0.8353 | 0.4354 | 468.25<br>371.30 | 0.0169<br>1.1487 | 0.0381<br>0.9289 | 0.4835 | 472.24<br>371.95 | 0.0152<br>1.1538 | 0.0343<br>0.9298 | 0.4820 |
| Lu6 | 373.11<br>365.68 | 0.0900<br>0.7394 | 0.1871<br>0.8177 | 0.5024 | 411.75<br>367.98 | 0.0231<br>1.1533 | 0.0517<br>0.9297 | 0.4907 | 406.82<br>368.65 | 0.0258<br>1.0004 | 0.0576<br>0.9000 | 0.4788 |

**Table 4a- Calculated maximum absorption wavelength ($\lambda_{max}$), vertical excitation energy, excited state oxidation potential, driving force of electron injection( $\Delta G^{inject}$ ),driving force of dye degeneration($\Delta G^{reg}$) of dyes at B3LYP/6- 311++G (d, p) level of theory in Gas Phase.**

| DYE | GAS PHASE | | | | |
|---|---|---|---|---|---|
| | $\lambda_{max}$ | $\lambda_{max}^{ICT}$ | $E_{ox}^{dye*}$ | $\Delta G^{inject}$ | $\Delta G^{reg}$ |
| **Lu** | 323.14 | 3.8368 | 2.5973 | -1.4027 | 1.5841 |
| **Lu1** | 400.51 | 3.0957 | 2.0181 | -1.9819 | 0.2638 |

| | | | | | |
|---|---|---|---|---|---|
| **Lu2** | 365.95 | 3.3882 | 2.6163 | -1.3837 | 1.1545 |
| **Lu3** | 400.93 | 3.0924 | 2.3216 | -1.6784 | 0.5604 |
| **Lu4** | 379.12 | 3.2703 | 2.0863 | -1.9137 | 0.5066 |
| **Lu5** | 407.05 | 3.0459 | 2.3060 | -1.6940 | 0.5019 |
| **Lu6** | 373.11 | 3.3230 | 2.2352 | -1.7648 | 0.7082 |

**Table 4b- Calculated maximum absorption wavelength ($\lambda_{max}$), vertical excitation energy, excited state oxidation potential, driving force of electron injection( $\Delta G^{inject}$ ),driving force of dye degeneration($\Delta G^{reg}$) of dyes at B3LYP/6- 311++G (d, p) level of theory in solvent Phases.**

| | DMF | | | | | | DCM | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DYE | $\lambda_{max}$ | $\lambda_{max}^{ICT}$ | $E_{ox}^{dye*}$ | $\Delta G^{inject}$ | $\Delta G^{reg}$ | $\lambda_{max}$ | $\lambda_{max}^{ICT}$ | $E_{ox}^{dye*}$ | $\Delta G^{inject}$ | $\Delta G^{reg}$ |
| **Lu** | 347.62 | 3.5666 | 2.8817 | -1.1183 | 1.5977 | 350.00 | 3.5424 | 2.9018 | -1.0982 | 1.5942 |
| **Lu1** | 408.53 | 3.0349 | 2.0436 | -1.9564 | 0.2285 | 417.12 | 2.9724 | 2.859 | -1.1410 | 0.9814 |
| **Lu2** | 406.26 | 3.0519 | 2.8317 | -1.1683 | 1.0336 | 400.85 | 3.0930 | 2.7925 | -1.2075 | 1.0355 |
| **Lu3** | 462.61 | 2.6801 | 2.7856 | -1.2144 | 0.6157 | 466.16 | 2.6597 | 2.7818 | -1.2182 | 0.5915 |
| **Lu4** | 479.01 | 2.5883 | 2.8117 | -1.1882 | 0.5501 | 482.77 | 2.5682 | 2.8079 | -1.1921 | 0.5261 |
| **Lu5** | 407.05 | 3.0459 | 2.3822 | -1.6178 | 0.5781 | 472.24 | 2.6254 | 2.7755 | -1.2245 | 0.5509 |
| **Lu6** | 411.75 | 3.0112 | 2.5043 | -1.4957 | 0.6655 | 406.82 | 3.0476 | 2.4583 | -1.5417 | 0.6559 |

**Table 5- The open-circuit Voltage ($V_{OC}$) and electron coupling constants ($|V_{RP}|$)in eV.**

| DYE | GAS PHASE | | DMF | | DCM | |
|---|---|---|---|---|---|---|
| | $V_{OC}$ | $V_{RP}$ | $V_{OC}$ | $V_{RP}$ | $V_{OC}$ | $V_{RP}$ |
| **Lu** | 1.8277 | 1.2170 | 1.7233 | 1.2238 | 1.7409 | 1.2221 |
| **Lu1** | 1.6640 | 4.5569 | 1.6270 | 0.5392 | 1.5420 | 0.9157 |
| **Lu2** | 0.9970 | 1.0022 | 0.9970 | 0.9418 | 1.0264 | 0.9427 |
| **Lu3** | 1.6054 | 0.7052 | 1.6019 | 0.7328 | 1.6043 | 0.7207 |
| **Lu4** | 1.0639 | 0.6783 | 0.8987 | 0.7000 | 0.9129 | 0.6880 |
| **Lu5** | 1.6177 | 0.6759 | 1.6033 | 0.7140 | 1.6065 | 0.7004 |
| **Lu6** | 1.2386 | 0.7791 | 0.9910 | 0.7577 | 1.0207 | 0.7529 |

**Table 6 - The ionization potential (IP) electron affinity (EA), chemical potential (μ), chemical hardness (η), softness(S), electrophilicity(ω), electron donating power (ω-), and electron accepting power (ω+) in eV calculated at B3LYP/6-311++ G (d, p) level of theory.**

| DYE | IP | EA | μ | η | S | ω | $\omega^+$ | $\omega^-$ |
|---|---|---|---|---|---|---|---|---|
| **Lu** | 6.4341 | 2.1723 | 4.3032 | 2.1309 | 0.4692 | 4.3450 | 2.4597 | 6.7629 |
| **Lu1** | 5.1138 | 2.3362 | 3.7250 | 1.3888 | 0.7200 | 4.9955 | 3.3085 | 7.0356 |
| **Lu2** | 6.0045 | 2.7766 | 4.3905 | 1.6139 | 0.6196 | 5.9720 | 3.9784 | 8.3689 |
| **Lu3** | 5.4104 | 2.3946 | 3.9025 | 1.5079 | 0.6631 | 5.0499 | 3.2871 | 7.1896 |
| **Lu4** | 5.3566 | 2.9361 | 4.1463 | 1.2102 | 0.8263 | 7.1028 | 5.1808 | 9.3272 |
| **Lu5** | 5.3519 | 2.3823 | 3.8671 | 1.4848 | 0.6734 | 5.0358 | 3.2879 | 7.1550 |
| **Lu6** | 5.5582 | 2.7614 | 4.1598 | 1.3984 | 0.7151 | 6.1870 | 4.2819 | 8.4417 |

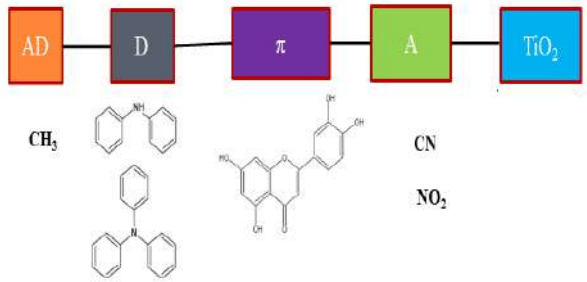



**Figure 1. Different parts of AD-D-π-A system. AD = auxiliary donor, D = donor, π = pi-spacer, A = acceptor**

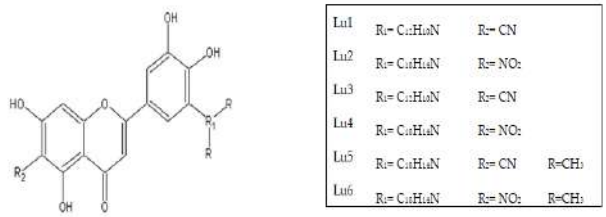



**Figure 2. Chemical structure of newly designed dyes.**

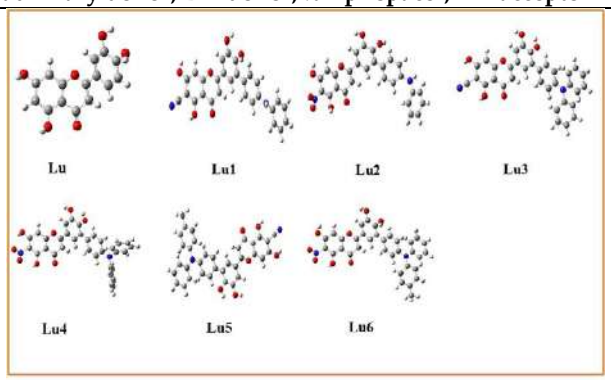



**Figure 3. Optimized geometrical Structure of the designed dyesat B3LYP/6-311++ G (d, p) level of theory in gas Phase.**

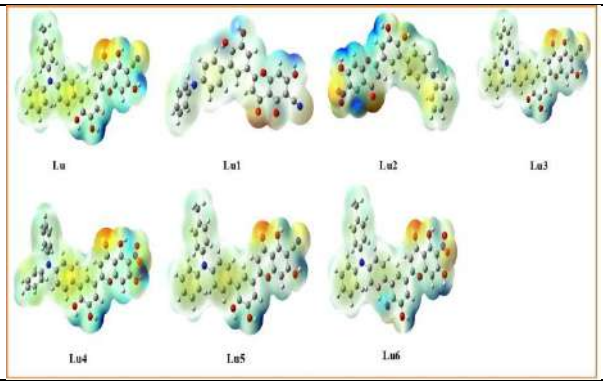



**Figure 4. Molecular electrostatic potential diagram of the designed dyesat B3LYP/6-311++ G (d, p) level of theory in gas Phase.**

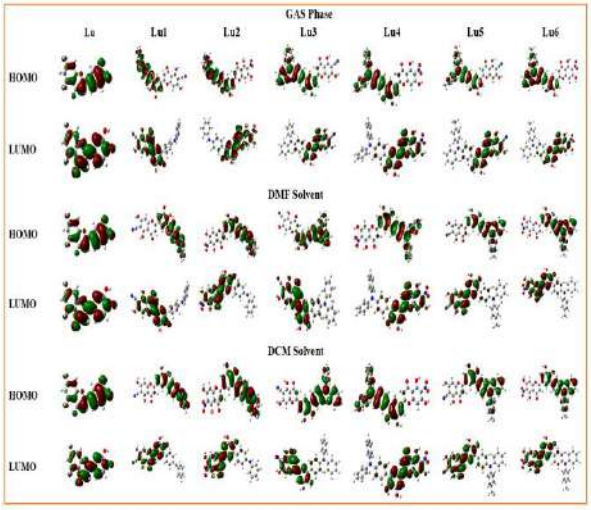



**Figure 5. HOMO and LUMO distribution pattern of Luteolin based dyes at DFT/B3LYP/6-311++G (d, p) level of theory in gas phase.**

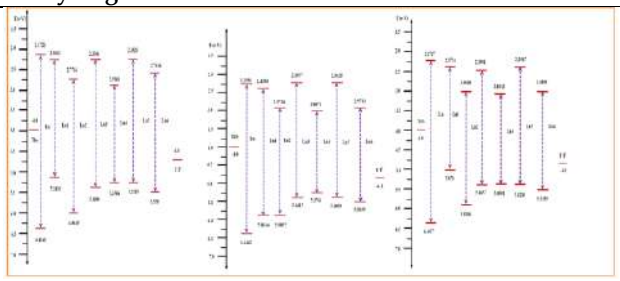
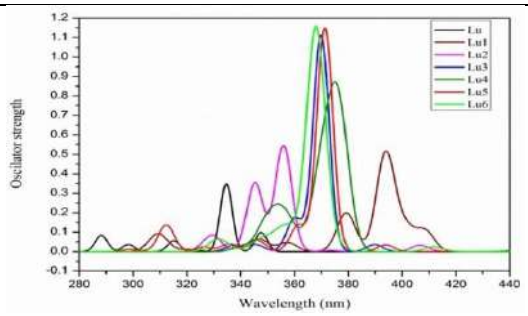
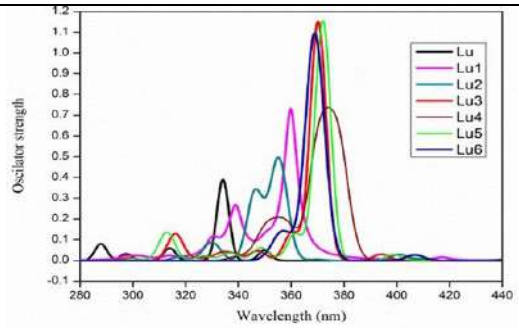


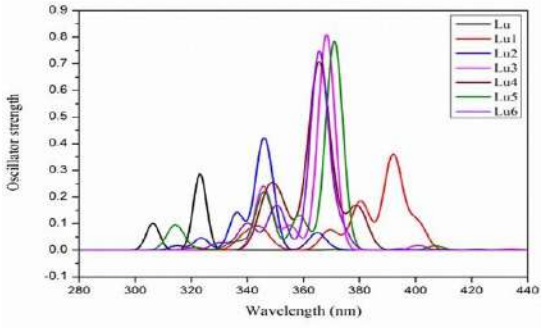
**Figure 6. HOMO, LUMO energies and energy gap of Luteolin and designed dyes in Gas Phase, DMF and DCM solvent.**




**(a)**




**(b)**




**(c)**

**Figure 7. Stimulated UV-visible absorption spectra of dyes calculated in (a) gas phase (b) DMF (c) DCM at TD-DFT / B3LYP /6-311++ G (d, p) level of theory.**

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**RESEARCH ARTICLE**

# Factors Determining Energy Consumption of Urban Households in Madurai District

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**ABSTRACT**

Energy consumption in urban households is critical for modern life, powering homes, workplaces, and transportation. However, many overlook the social, environmental, and economic consequences of energy use. This study focuses on Madurai district, Tamil Nadu, India, where understanding household energy consumption patterns is vital for promoting sustainable practices. The research explores the key factors influencing energy use in Madurai's urban dwellings. Understanding these drivers is crucial for crafting targeted policies and promoting responsible consumption habits. This knowledge empowers individuals and communities to make informed decisions for a more sustainable future. The study's significance extends beyond Madurai. Its findings can serve as a valuable case study for other urban centers facing similar challenges. By uncovering the factors behind household energy consumption, this research aims to empower policymakers, urban planners, and residents to work together towards a sustainable energy future for Madurai and similar cities.

**Keywords :** Energy, Urban, Household, Sustainable and Consumption

## INTRODUCTION

The term "energy" comes from the Greek word "energeia," which meaning "working." It is the ability or power to work. The capacity or ability to work is defined as energy. The Joule is a unit of energy measurement. Energy is defined as the ability to work and overcome obstacles in physics. This word is a synonym for strength, energy, and

might. Anything that moves or is made to move is under the influence of a motivational force. It's termed 'energetic' in short. Working, walking, running, climbing a stairway, or lifting a log of wood all takes energy. Energy can be dormant or latent. However, while seeking energy to satisfy their needs; many people give little or no consideration to the social, environmental, and economic impacts of its uses. It powers our homes, workplaces, and transportation. While urban households rely heavily on energy for their daily needs, understanding the factors that influence their consumption patterns is crucial for promoting sustainable practices. This study explores the key determinants of energy use in Madurai district's urban households.

Energy, the lifeblood of our modern world, pulsates through our homes, workplaces, and transportation systems. Urban households, the beating heart of Madurai's economy, are particularly reliant on this invisible force to illuminate their lives, power their appliances, and fuel their kitchens. Understanding the determinants of energy consumption in Madurai's urban dwellings is not merely an academic exercise. It holds the key to unlocking a more sustainable future for the district. By identifying the drivers behind energy use, it can craft targeted policies and promote responsible consumption practices. This knowledge empowers us to make informed decisions, not just on an individual level, but also at a community-wide scale. This exploration not only holds significance for Madurai but also serves as a valuable case study for other urban centers grappling with similar challenges. This quest for knowledge and believe the insights garnered will empower policymakers, urban planners, and residents alike to collectively chart a course towards a more sustainable energy future for Madurai. The objectives of the study are to study the socio-economic conditions and the energy consumption pattern of households in the study areas and to suggest suitable measures to solve the problem of energy and reduce environmental pollution

## REVIEW OF LITERATURE

Filippin. C., *et al.* (2019) had made an attempt to study on "Analysis of energy consumption patterns in multi-family housing in a moderate cold climate" in La Pamba, Argentina. The study found that first floor of each block showed less natural consumption and greater share of gas for heating purposes, the winter energy consumption of the multi family dwelling(200-400 $m^3$) is lower than that of a single family dwelling (500$m^3$). Flats receiving solar contribution from the north consume 36 per cent less energy for heating than the south. It was found that 90 per cent energy consumed was natural gas and the flats on the top floor face south and on the ends consumed more energy due to less availability of solar resources to heat and light.

Miah Danesh *et. al* (2020), conducted a study on, "Rural Household Energy Consumption Pattern in the Disregarded Villages of Bangaladesh", The objective of the study was to assess the overall energy consumption pattern of the households in rural areas and its influencing factors. There was a significant relation between per capita household expenditure for energy consumption and total income at both $p < 0.01$ and $p < 0.05$ significance levels. The study found that positive relationship between growth in income and household demand for commercial fuels and no significant relationship between monthly total household expenditure for biomass consumption and total income and also when the income of the household increases, they wish to shift from traditional energy use system to modern energy system.

## METHODOLOGY

The study is exploratory in nature relying on both primary and secondary data. For collection of primary data survey method is adopted. Survey is conducted through questionnaire that is designed taking into consideration pre tested questionnaire after making modification to make the questionnaire suitable for the present study. The secondary data regarding the number of households in the study area have been collected from the records of District Statistical Hand Book of Madurai District. Further, data from Census 2011, BP statistical Review of World Energy – February 2024 and Economic Survey 2022 -2023 and Tamil Nadu Electricity Board are also collected for the study. Personal

interviews and unbiased observations are the most suitable methods to establish a good rapport with the respondents to collect necessary data. For studying about a social problem it is difficult to study from the whole universe. This is because it is costly, time consuming and complex as well as financially not viable. It is therefore convenient to pick up a sample out of the universe proposed to be covered by the study. The information collected from the sample units are with reference to the financial year from April 2023 to March 2024. Percentage Analysis used for this study.

## RESULT AND DISCUSSION

Thus this paper discussed the socio-economic background of the sample household's respondents in urban areas and the energy consumption pattern of households.

**Vehicles Owned by the Respondents :** Now-a-days the users of vehicle population in urban areas tremendously increased. The increased vehicle leads to a greater demand for commercial fuels like petrol and diesel. Moreover, it pollutes the quality of air and it has its own impact on the health of the people. In this connection, the researcher wants to know about the vehicles owned by the sample respondents in the study area accordingly. It is clear from Table 2 that in 279 (93.00 per cent) households have two wheelers of different kinds. In urban areas all the sample households have two wheelers, 67 (37.22 per cent) households own cars and only seven (3.89 per cent) households own non – fuel vehicles. It is concluded that majority of the respondents in urban areas having own two wheelers to carry out their daily activities.

**Nature of Lighting Equipments :** The different types of electrical equipments are used by the sample respondents according to their utility. The energy consumption of lighting equipments vary according to the nature of lighting equipments like tube lights, CFL, incandescent lamps, LED *etc.* Tube lights and incandescent lamps are consume more energy than CFL and LED bulbs. But the use of CFL and LED bulbs are not good in number. In this connection, the researcher wants to estimate their nature of electrical equipments used by the sample respondents in the study areas. Accordingly the researcher collected information. Table 3 clearly depicts the nature of lighting equipments used by the sample respondents in the study areas. Regarding urban areas almost all the households are predominantly using tube lights. Still in urban areas 140 (77.78 per cent) are using incandescent lamps. CFLs are used by 115 (63.89 per cent) respondents. Twenty two respondents who use only LED bulbs. It is concluded from this table that the use of CFLs and LED bulbs are lesser in numbers and the respondents who use these bulbs are only addition to the tube lights and incandescent lamps.

**Home Appliances Usage of Households :** Due to the development of science and technology the different varieties of home appliances are manufactured and innovated for public use. The use of home appliances is one of the indicators of development. Table 4 explains the different types of home appliances used by the sample respondents in the study areas.

The different types of home appliances used by the sample respondents in the study areas are fan, television, radio, ion box, mixie, grinder, refrigerator, washing machine, heater, air conditioner, computer, electric stove *etc.* according to their life style and standard of living. It is evident from Table 4.14 that fan and TV are used by all the households irrespectively. But the number of fans users vary washing machines are mostly used in urban areas. Out of 180 respondents, 165 (91.67 per cent) respondents use machine for washing clothes, it is only five (1.67 per cent) in areas. Air conditioner and electric stove users is again high in urban areas. Major types of fuel used by the households in the sample areas for cooking is classified by the researcher as firewood and agricultural waste, kerosene and LPG. The researcher collected and classified the information regarding energy for cooking in the study areas and the same is shown in Table 5.

It is understood from Table 5 that sample households who are users of multi fuels for cooking. considering the urban areas, the sample respondents are using only two of fuels namely kerosene and LPG. Kerosene is consumed by 42 (23.33 per cent) respondents and LPG is used by all the 180 (100.00 per cent) sample respondents. Fire wood usage is nil in urban areas.

**Fuel Usage for Transportation by the Households :** The researcher gathered information about energy usage for transportation in the study area by the household respondents.

It is evident from Table 6 that in urban area 155 (86.11 per cent) households are using petrol consuming vehicles and 25 (13.89 per cent) households are using diesel consuming vehicles.

**Expenditure Pattern by Urban Households :** The expenditure pattern of the sample urban household is estimated in the form of minimum, maximum and average. It is clear form Table 7 that in the urban areas the money spent on energy is higher compared to rural areas. Further, money spent on energy is greater than money spent on education and medical. The average amount of money spent to meet their food requirement is calculated as Rs. 5850 per month. The average amount of expenditure made per month by the sample respondents on cloth and rent is Rs. 1240 and Rs. 3200 respectively. Entertainment expenditure and social gathering expenditures are high in urban areas. The average amount estimated is Rs. 2445 and Rs. 1760 for social gathering and entertainment respectively. The money spent on energy per month is estimated and the values are Rs. 450, Rs. 4500 and Rs. 2450 as minimum, maximum and average respectively.

## CONCLUSION

The take a look at on an economic observe of intake sample of energy inside the household sectors and its impacts in Madurai district of Tamil Nadu ought to pave the way for different researchers to adopt such fashion of comparable research in other regions. Probably studies can be contemplated to some other places. Even the present look at can also help the coverage makers to adopt a suitable coverage to solve the problem of power consumption and also to conserve electricity viably. In energy as a source of electricity accompanied by way of LPG in both Madurai cities. Regarding zone-sensible energy expenditure in west quarter, kerosene changed into found to be maximum while, Madurai metropolis, a most quantity turned into spent for power accompanied through kerosene. There existed a tremendous difference in quarter-smart energy consumption expenditure for kerosene and LPG in social area between Madurai metropolis.

## SUGGESTIONS

Based on the findings of the study, the following constructive and practicable recommendations are offered to minimize the problem of energy consumption and its impact on households in Madurai District.

- Rajiv Gandhi Grammeen Vidyutikaran Yojana (RGGVY), 2009 – 10 has set a goal to provide clean cooking energy such as LPG, NG, biogas or kerosene to all within 10 years. Hence, the Government may implement subsidized LPG cylinders to the rural people.The usage of bio-gas, smokeless chullas should be encouraged and awareness campaign may also be conducted regularly.
- To develop sustainable energy supply, women's groups can form oil seed plantations or tree – growing co-operatives to manage and produce bio- fuel and fuel wood with the same effort that they put into searching and gathering fuel wood today. Finance through self-help groups should be provided to transform women.
- The best way for providing subsidy for electricity and cleaner fuels, kerosene or LPG is to give an entitlement to the targeted households equivalent to 30 units of power and 6 kg of cooking gas or equivalent amount of kerosene to cover one or more needs. A system of debit cards or smart cards may be introduced whereby the targeted households get a credit of different amounts of cash for the purchase of these entitlements.
- To minimize fuel usage in transport small distance travels should be undertaken by walk or by bi-cycle.
- To Switch to cleaner Fuels

- There is enormous scope for energy conservation by adopting Energy-Efficient Lighting by replacing CFL or LED bulbs in place of incandescent bulbs which are not only energy efficient but also environment friendly.
- Awareness should be created through mass media regularly to use modern fuels that is LPG and electricity and also, to use Energy - Efficient Household Appliances. This will change the peoples' attitude towards modern fuels in due course of time. Apart from cooking and lighting, electrical appliances there are major sources of household energy consumption, especially in higher-income of urban households. The share of electrical appliances in the household energy consumption is likely to increase significantly in the future because of growth in per capita income.
- Eco friendly technologies such as solar cooker, solar heaters should be produced and made available at reasonable and affordable rates for the use of common people.

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**Table 1 : Socio-Economic Characteristics of the Sample Respondents in Urban Areas**

| Particulars | | Number of Respondents |
|---|---|---|
| Age (in Years) | 25 – 35 | 49 (27.22) |
| | 35 – 45 | 47 (26.11) |
| | 45 – 55 | 35 (19.44) |
| | 55 – 65 | 37 (20.56) |
| | 65 – 75 | 12 (6.67) |
| | **Total** | **180 (100.00)** |
| Sex | Male | 174 (96.67) |
| | Female | 6 (3.33) |
| | **Total** | **180 (100.00)** |
| Religion | Hindu | 160 (88.89) |
| | Christian | 11 (6.11) |
| | Muslim | 9 (5.00) |
| | **Total** | **180 (100.00)** |
| Community | OC | 6 (3.33) |
| | BC | 120 (66.67) |
| | MBC | 33 (18.33) |

| | | |
|---|---|---|
| | SC / ST | 21 (11.67) |
| | **Total** | **180 (100.00)** |
| Education | Primary | 15 (8.33) |
| | Secondary | 33 (18.33) |
| | Higher Secondary | 42 (23.34) |
| | Graduate | 49 (27.22) |
| | Post Graduate | 26 (14.45) |
| | Professional | 15 (8.33) |
| | **Total** | **180 (100.00)** |
| Occupation | Agricultural Collie | 7 (3.89) |
| | Private | 50 (27.77) |
| | Government | 51 (28.34) |
| | Business | 72 (40.00) |
| | **Total** | **180 (100.00)** |
| Income (in Rs.) | Up to 20000 | 52 (28.89) |
| | 20000 – 40000 | 87 (48.34) |
| | 40000 – 60000 | 31 (17.22) |
| | 60000 – 80000 | 6 (3.33) |
| | 80000 and above | 4 (2.22) |
| | **Total** | **180 (100.00)** |
| Type | Joint Family | 20 (11.11) |
| | Nuclear Family | 160 (88.89) |
| | **Total** | **180 (100.00)** |
| Family Members | 2 Members | 31 (17.22) |
| | 3 Members | 57 (31.67) |
| | 4 Members | 76 (42.22) |
| | 5 Members | 16 (7.78) |
| | **Total** | **180 (100.00)** |
| Nature of House | Pucca House | 118 (65.56) |
| | Semi – pucca House | 42 (23.33) |
| | Thatched House | 20 (11.11) |
| | **Total** | **180 (100.00)** |
| Number of Rooms | Only one Room | - |
| | Two Rooms | 4 (2.22) |
| | Three Rooms | 97 (53.89) |
| | Four Rooms | 41(22.78) |
| | Five Rooms | 26 (14.44) |
| | More Than Five Rooms | 12 (6.67) |
| | **Total** | **180 (100.00)** |

Source: Survey Data.
Note: Figures in brackets represent percentage to the total.

**Table 2 : Vehicles Owned by the Respondents**

| Vehicle | Number of Respondents |
|---|---|
| Car | 67 (37.22) |
| Two Wheeler | 180 (100.00) |
| Non – Fuel Vehicles | 7 (3.89) |

**Table 3 : Nature of Lighting Equipments**

| Equipment | Number of Respondents |
|---|---|
| Tube Light | 180 (100.00) |
| CFL | 115 (63.89) |
| Incandescent | 140 (77.78) |
| LED | 22 (12.22) |

Source: Survey Data.

**Table 4 : Home Appliances Usage of Households**

| Equipment | Number of Respondents |
|---|---|
| Fan | 180 (100.00) |
| TV | 180 (100.00) |
| Radio | 13 (7.22) |
| Iron Box | 132 (73.33) |
| Mixie | 176 (97.78) |
| Grinder | 171 (95.00) |
| Refrigerator | 174 (96.67) |
| Washing Machine | 165 (91.67) |
| Heater | 94 (52.22) |
| AC | 58 (32.22) |
| Computer | 96 (53.33) |
| Electric Stove | 66 (36.67) |

Source: Survey Data

**Table 5 : Fuel Used for Cooking in the Households**

| Fuel Type | Number of Respondents |
|---|---|
| Firewood and Agricultural Waste | - |
| Kerosene | 42 (23.33) |
| LPG | 180 (100.00) |

Source: Survey Data.

**Table 6 : Fuel Usage for Transportation by the Households**

| Fuel Type | Number of Respondents |
|---|---|
| Petrol | 155 (86.11) |
| Diesel | 25 (13.89) |

Source: Survey Data.

**Table 7 : Expenditure Pattern by Urban Households**

| Items | Minimum | Maximum | Average |
|---|---|---|---|
| Food | 4300 | 7800 | 5850 |
| Cloth | 1200 | 3000 | 1240 |
| Rent | 2500 | 5500 | 3200 |
| Education | 500 | 3000 | 1305 |
| Medical | 300 | 4000 | 2150 |
| Social Gathering | 500 | 3500 | 2445 |
| Energy | 450 | 4500 | 2450 |
| Entertainment | 200 | 3000 | 1760 |
| Miscellaneous | 500 | 3500 | 1935 |

Source: Survey Data.

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**RESEARCH ARTICLE**

# Evaluating the Efficacy of Alum and Spirulina for Wastewater Remediation : A Comparative Analysis

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## ABSTRACT

The unregulated release of wastewater laden with suspended solids poses a grave threat to natural water ecosystems, escalating pollution levels and imperilling aquatic life. Turbidity, crucial in water treatment, especially for potable water, emerges as a significant concern. While chemical treatments effectively reduce turbidity, their by-products contribute to environmental pollution and ecosystem degradation. This study compares *Spirulina* and alum in wastewater treatment, focusing on their impact on chemical parameters and water quality. Spirulina's nutrient absorption abilities and alum's conventional coagulation were evaluated for their potential in wastewater remediation. *Spirulina* notably reduced nitrate levels to 4.6 mg/L, surpassing untreated wastewater and tap water, showcasing its prowess in nitrogen removal. Additionally, it effectively decreased chloride to 0.059556 mg/L and significantly raised dissolved oxygen to 246.30 mg/L, crucial for aquatic life support. Alum treatment, while less pronounced, showed commendable reductions in nitrate and chloride and improved dissolved oxygen (8.92 mg/L, 0.4565 mg/L, and 94.58 mg/L, respectively). Both treatments mitigated phosphate, with *Spirulina* achieving a lower concentration (539,215.68 mg/L) than alum (8,400 mg/L). Overall, *Spirulina* exhibited slightly superior efficacy in reducing nitrate and chloride and significantly enhancing dissolved oxygen, while alum excelled in phosphate removal. Choosing between them should consider specific pollutant removal goals and operational factors, with *Spirulina* favored for overall pollutant reduction and enhanced oxygenation, and alum for efficient phosphate removal.

**Keywords:** wastewater treatment, Spirulina platensis, alum, chemical parameters, water quality enhancement, sustainable water management.

## INTRODUCTION

Water, as a vital resource essential for sustaining life on Earth, faces growing threats to its availability and quality due to human activities and environmental deterioration. The issue of turbidity, which refers to the presence of suspended particles clouding the water, emerges as a significant obstacle in efficiently managing water quality standards. Beyond diminishing the visual clarity of water bodies, turbidity also jeopardizes the safety and usability of water for a variety of essential purposes. Given these challenges, it becomes imperative to implement robust and innovative water treatment techniques to effectively address turbidity and mitigate the associated health and environmental risks. In doing so, we can safeguard both the quantity and quality of our precious water resources, ensuring their sustainable use and resilience in the face of ongoing anthropogenic pressures and environmental stressors.

Coagulation-flocculation processes are essential steps in water treatment as they play a crucial role in the removal of suspended particles and the reduction of turbidity levels in water sources. The use of traditional coagulants like alum (aluminium sulfate) has been prevalent in the industry due to their established effectiveness in destabilizing particles and promoting aggregation. Despite their widespread application, there are growing concerns surrounding the environmental and health implications associated with residual aluminium content in treated water, prompting a shift towards exploring alternative coagulant options within the water treatment sector. By expanding the scope of research and development in this area, industries are actively seeking alternative coagulants that can achieve similar, if not improved, treatment outcomes while mitigating potential risks related to aluminium exposure. This shift towards innovation underscores a commitment to safeguarding water quality standards and ensuring the sustainability of water treatment practices in the face of evolving environmental considerations and health priorities.
Most particulate matter, such as fine particles, remains suspended due to their inability to settle by gravity, a characteristic that allows them to easily pass through the pores of typical filtration media. Traditionally, the challenge of entrapping and eliminating colloids in water has been addressed through coagulation processes, often utilizing chemical coagulants like alum. However, in many developing nations, this treatment approach is deemed unviable due to its substantial costs and the complexities associated with evaluating chemical coagulants, particularly alum. Typically, the chemicals employed in diverse treatment systems consist of synthetic organic and inorganic compounds. The drawback lies in their high expenses, driven by the need for relatively larger doses, thereby diminishing their cost-effectiveness in most scenarios.

One notable alternative that holds promise for addressing turbidity issues in water treatment is *Spirulina*, a type of filamentous cyanobacterium highly esteemed for its exceptional nutritional content and eco-friendly characteristics. The remarkable coagulation abilities of Spirulina are primarily attributed to the presence of polysaccharides and proteins within its cellular structure. These attributes make Spirulina a highly viable option for enhancing the process of turbidity removal in water treatment systems. Numerous scientific investigations have highlighted how Spirulina efficiently interacts with suspended particles by forming strong bonds with them. This interaction aids in the subsequent removal of these particles either by sedimentation or through filtration mechanisms. Moreover, Spirulina's inherent biodegradable nature and non-toxic properties further accentuate its suitability as a sustainable solution for enhancing water quality and promoting environmental conservation efforts.

Upon reviewing the existing body of literature, it becomes apparent that a significant amount of research effort has been dedicated to studying the effectiveness of Spirulina as a coagulant for removing turbidity in water treatment processes. Interestingly, a study by Okolie and colleagues in 2019 not only compared Spirulina with traditional alum but also underscored Spirulina's comparable efficiency in removing turbidity while also hinting at its potential to serve as a sustainable and eco-friendly substitute for alum. This finding was further supported by Eze et al. in 2020, who observed a noteworthy decrease in turbidity levels in water treated with Spirulina, emphasizing its efficacy in this regard and the environmental advantages it presents.

Looking beyond its turbidity removal capabilities, Spirulina showcases additional benefits that make it a versatile player in water treatment technologies. For instance, a study by Ghasemi et al. in 2018 delved into *Spirulina's* ability to target organic matter in water, suggesting a broader scope of application for this natural coagulant. Furthermore, investigations by Sathishkumar and NCBI and their respective teams in 2019 and 2021 revealed Spirulina's potential in removing heavy metals from water sources, highlighting its adaptability in tackling diverse water quality challenges effectively. As such, the shift towards utilising environmentally friendly natural coagulants, like Spirulina, presents itself as a promising alternative for treating contaminated surface water, offering both economic and operational efficiency benefits, especially at the household level. Incorporating natural coagulants alongside synthetic ones can not only yield cost savings by reducing chemical expenses but also work towards minimizing potential threats associated with extensive chemical usage in water treatment processes. Ultimately, this balanced approach towards water treatment stands to enhance overall efficiency while promoting sustainability in managing water resources.

**Need for the Study**

The use of natural coagulants, including Spirulina, for water treatment purposes can provide a cost-effective solution that yields treated water with stable pH levels and is highly biodegradable. These natural coagulants are generally considered safe for human health, offering a sustainable alternative to commercial counterparts. Unlike some synthetic coagulants that are effective only within specific pH ranges, natural coagulants like Spirulina can facilitate efficient flocculation processes in a broader spectrum of water conditions. This increased versatility in treatment capabilities highlights the benefits of incorporating natural coagulants into water treatment practices, promoting both environmental sustainability and improved water quality outcomes.

**Scope of the Study**

This scientific work aimed to address the global issue of decreasing fish populations due to environmental impacts caused by untreated effluent disposal. By focusing on the development of a method using *Spirulina* and alum-treated water for fish culture, the researchers sought to create a sustainable solution. Alum, known for its ability to clarify cloudy water in aquariums by collecting suspended particles, was utilized cautiously to avoid potential harm from excess aluminium. Furthermore, Spirulina was identified as an economical medium for biomass production, aiding in the reduction of certain inorganic nutrients. By exploring the use of environmentally friendly natural coagulants, this study proposed a practical alternative for treating contaminated surface water at a domestic level. Through the combination of natural and synthetic coagulants, the work aimed to not only reduce chemical costs but also minimize associated threats effectively.

## MATERIALS AND METHODS

## MATERIALS

**Conventional Coagulant**

In this Study, Aluminium sulphate (Alum) was used as a conventional coagulant and the stock solution (1 % strength) was prepared by adding 10 grams of Alum to 1000 ml of distilled water.

**Natural Coagulants**

The natural coagulants such as*Spirulina*, used in this study were depicted in Figure 1

**Material required for *Spirulina* growth curve.**

BG-11 medium was purchased from Himedia, India. *Spirulina*, Conical flasks, Measuring cylinder, sterile test tubes, and Distilled water.

**COLLECTION OF WATER SAMPLES**

The required amount of water samples was collected in sterile cans from different days of form home kitchen for analysis and the cans used were rinsed with the same sample before collection.

## METHODOLOGY

**Isolation of *Spirulina platensis***

Water samples from freshwater ponds in Trichy District, India, were used to extract *Spirulina.*The samples were placed in sterile BG 11 medium in Erlenmeyer flasks and incubated in a growth chamber set at 3000 lux light intensity, 16 hours light exposure, 8 hours darkness, and 28°C. Algal growth was monitored for Spirulina platensis development. Once confirmed, isolates were preserved on BG 11 medium for further research.

**Growth curve analysis of Spirulina**

In the experiment, Spirulina algae were grown in a carefully prepared BG 11 medium under controlled conditions (28 ± 2 _C temperature, 52–55 mol photon m-2 s-1 light intensity, and 16:8 light: dark cycles). Growth was monitored by regularly testing 1 ml samples from the culture, measuring their optical density at 620 nm with a spectrophotometer. When the readings stabilized, they were analyzed collectively to create a growth curve, illustrating the algae's development over time and aiding in understanding its growth dynamics.

**Analysis of Physiochemical Parameters of Wastewater**

**Wastewater collection**

Grey water (kitchen wastewater) samples were collected with the aid of 25 litters capacity of plastic container from the point of sink tank of house, Rajapalyam.

**Water Quality Analysis**

**Physical parameters**

Temperature and Light Intensity were measured using Thermometer and Lux meter at different intervals (10 am, 1 pm, and evening 4 pm).

**Chemical parameters**

pH of the water sample was determined by using Digital pH meter. Every day the pH of the samples was measured two times one at 10.00am and at 5.00 pm.The following water quality parameters such as TSS, TDS, $CO_3$, Na , Mg, Fe, Ca, $Po_4$, K, S and total alkalinity were measured. The above water quality parameters except temperature, light intensity and pH, the other parameters were tested.

**Comparison Study of Remediation of Wastewater Using Spirulinaand Alum**

To compare the efficacy of *Spirulina* and alum in the remediation of wastewater, two separate treatment systems were established. Raw wastewater was collected from a kitchen wastewater treatment and characterized for baseline parameters including pH, turbidity, chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solids (TSS). For the Spirulina treatment. The concentration of Spirulina was maintained at 1 g/L. For the alum treatment, aluminium sulfate was dosed at 50 mg/L, and the mixture was subjected to rapid mixing for 1 minute followed by slow mixing for 20 minutes and a settling period of 30 minutes. Both treatments were monitored over a period of 30 days. Water samples were collected at 24-hour intervals and analysed for changes in pH, turbidity, COD, BOD, and TSS. The efficiency of each treatment method was assessed by comparing the percentage reductions in these parameters.

## RESULT

**Isolation of *Spirulina***

Water samples from freshwater ponds in Trichy District, India, were utilized for isolating Spirulina. These samples were inoculated into 100 ml of sterile BG 11 medium contained in Erlenmeyer flasks. The inoculated flasks were then incubated in a growth chamber designed to maintain a light intensity of 3000 lux, with a 16/8-hour light-dark cycle at a constant temperature of 28°C. Periodic examination of the flasks revealed algal growth, which was subsequently isolated and identified microscopically as Spirulina. The identified Spirulina platensis cultures were successfully maintained on BG 11 medium.

***Spirulina* growth curve analysis**

The optical density (OD) measurements of the Spirulina sample were recorded at various time intervals to monitor its growth. Initially, the OD value was 0.05. After 6 hours, the OD increased to 0.18, indicating the beginning of active growth. At the 12-hour mark, the OD further increased to 0.24. A significant growth spurt was observed by 24 hours, with the OD reaching 0.35. The highest OD value recorded was 0.49 at 48 hours, reflecting peak growth. By 72 hours, the OD decreased to 0.32, suggesting a decline in growth rate or the onset of a stationary phase. These observations highlight the dynamic growth pattern of Spirulina in the given conditions. The optical density (OD) measurements of the Spirulina sample were recorded at various time intervals to monitor its growth. Initially, the OD value was 0.05. After 6 hours, the OD increased to 0.18, indicating the beginning of active growth. At the 12-hour mark, the OD further increased to 0.24. A significant growth spurt was observed by 24 hours, with the OD reaching 0.35. The highest OD value recorded was 0.49 at 48 hours, reflecting peak growth. By 72 hours, the OD decreased to 0.32, suggesting a decline in growth rate or the onset of a stationary phase. These observations highlight the dynamic growth pattern of Spirulina in the given conditions

**Water Quality Analysis**
**Physical parameters**

***Spirulina***

The analysis of wastewater treatment methods involving Spirulina reveals significant improvements across several parameters. Initially, the untreated wastewater exhibited undesirable characteristics, including a pH of 5.39, high TDS (1331 mg/l), elevated EC (2768 μS/cm), and a turbidity of 0.48 NTU, accompanied by a black color and noticeable odor. Post-treatment with Spirulina, there were notable improvements observed. The pH increased to 7.7, indicating a shift towards neutrality. TDS levels decreased to 667 mg/l, and EC reduced to 1980 μS/cm, signifying a significant reduction in dissolved solids and electrical conductivity. Turbidity also improved considerably to 0.30 NTU. Moreover, the water's color changed to light green, indicating a visual change in water quality. Importantly, the treatment succeeded in eliminating the previously present odor. These results highlight the effectiveness of Spirulina in enhancing water quality parameters, particularly in terms of pH adjustment, reduction of TDS and EC, and improvement in turbidity. However, the introduction of a light green color might be a consideration depending on the aesthetic requirements of the treated water. Overall, Spirulina treatment demonstrates promise as an environmentally friendly and sustainable solution for wastewater treatment, particularly in contexts where odor elimination and moderate color changes are acceptable.

**ALUM**

The pH level was measured at 4.64, indicating an acidic environment, which is characteristic of alum-treated water. The Total Dissolved Solids (TDS) were recorded at 527 mg/L, which suggests a moderate concentration of dissolved substances. Electrical Conductivity (EC) was found to be 1117 μS/cm, reflecting a higher ion concentration in the water. The turbidity was measured at 0.49 NTU, indicating a relatively low level of suspended particles, contributing to clearer water post-treatment. The parameters for colour and odour were not specified in this analysis. Overall, these parameters suggest that the alum treatment effectively reduces turbidity and maintains a moderate level of

dissolved solids, though the acidic pH may require further adjustment depending on the intended use of the treated water.

**Chemical parameters**

***Spirulina***

The chemical analysis of the Spirulina and wastewater treatment sample revealed the following results: The nitrate concentration was measured at 4.6 mg/L. Chloride levels were found to be 0.059556 mg/L, and magnesium was absent at 0 mg/L. The dissolved oxygen content was notably high at 246.30 mg/L, indicating a well-oxygenated environment. Calcium concentration was significantly elevated at 571.914 mg/L. Sodium and potassium levels were relatively low, measured at 0.1054 mg/L and 0.0068 mg/L respectively. The phosphate concentration was exceedingly high at 539,215.68 mg/L, while sulphate was present at 1 mg/L. Phosphorus was detected at 0.05 mg/L. These results suggest that the treatment process significantly alters the chemical composition of the wastewater, with particularly high levels of dissolved oxygen and phosphate, and elevated calcium, which may be beneficial for certain applications but could also indicate the need for further treatment to balance the chemical parameters for specific uses.

**ALUM**

The nitrate concentration was measured at 8.92 mg/L, indicating a moderate level of nitrates in the treated water. Chloride levels were found to be 0.4565 mg/L, while magnesium was absent at 0 mg/L. The dissolved oxygen content was 94.58 mg/L, which is adequate but lower compared to some other treatment processes. Calcium concentration was recorded at 159.7995 mg/L, and sodium at 0.222 mg/L. Potassium levels were 0.1362 mg/L. The phosphate concentration was significantly high at 8,400 mg/L, suggesting substantial phosphate presence in the treated water. Sulphate was present at 0.66 mg/L, and phosphorus at 0.05 mg/L. These results indicate that alum treatment impacts the chemical composition of wastewater by increasing nitrate and phosphate levels, while also maintaining moderate levels of other key ions. The elevated phosphate levels suggest a need for additional treatment to reduce them, depending on the intended use of the treated water.

The comparison of different wastewater treatment methods reveals significant variations in their effectiveness across various parameters. Initially, the untreated wastewater exhibited unfavourable characteristics, including a pH of 5.39, high TDS (1331 mg/l), elevated EC (2768 μS/cm), and a turbidity of 0.48 NTU, accompanied by a black color and noticeable odor. Control tap water, representing the benchmark for ideal water quality, demonstrated a pH of 7.91, significantly lower TDS (303 mg/l), reduced EC (614 μS/cm), minimal turbidity (0.02 NTU), and no color or odor. After treatment with Spirulina, the wastewater's pH increased to 7.70, approaching the neutral range. TDS levels decreased to 667 mg/l, and EC reduced to 1980 μS/cm, indicating a notable improvement in water purity. Turbidity also improved to 0.30 NTU, contributing to clearer water. The color changed to light green, indicating a visual change in water quality. Importantly, the treatment successfully eliminated the previously present odor.Alum treatment resulted in a pH of 4.64, indicating increased acidity. TDS levels reduced to 527 mg/l, and EC decreased to 1117 μS/cm. Turbidity remained at 0.49 NTU, comparable to the untreated water. While the color and odor were not specifically addressed in the treatment process, they were absent in the final product.

*Spirulina* treatment demonstrated remarkable efficacy in reducing nitrate levels to 4.6 mg/L, substantially lower than both untreated wastewater and tap water control, indicating its proficiency in nitrogen removal. Additionally, Spirulina treatment achieved a notable decrease in chloride concentration to 0.059556 mg/L, showcasing its ability to mitigate chloride contamination effectively. Dissolved oxygen levels surged to 246.30 mg/L after Spirulina treatment, indicating significant oxygenation, which is crucial for supporting aquatic life. Alum treatment also yielded commendable results, particularly in reducing nitrate levels to 8.92 mg/L and chloride concentration to 0.4565 mg/L. While not as drastic as Spirulina treatment, these reductions still signify effective pollutant removal. Dissolved oxygen levels post-Alum treatment reached 94.58 mg/L, indicating improved oxygenation, albeit to a lesser extent compared to Spirulina treatment.

Moreover, both treatments succeeded in reducing phosphate levels significantly. Spirulina treatment lowered phosphate concentration to 539,215.68 mg/L, while Alum treatment achieved a remarkable reduction to 8,400 mg/L.In terms of overall effectiveness, Spirulina treatment appears to be slightly more efficient in reducing nitrate and chloride levels compared to Alum treatment. Additionally, Spirulina treatment notably improved dissolved oxygen levels to a higher extent. However, Alum treatment showed notable effectiveness in reducing phosphate levels. Thus, while both treatments exhibit efficacy in improving water quality, the choice between Spirulina and Alum treatment may depend on specific contaminant removal goals and operational considerations. Spirulina treatment might be preferred for its comprehensive reduction of various pollutants and enhancement of dissolved oxygen levels, while Alum treatment could be favored for its efficiency in phosphate removal.

## DISCUSSION

The successful isolation and cultivation of Spirulina platensis from freshwater ponds in Trichy District, India, as described in this study, underscore the adaptability and potential of this microorganism. By inoculating water samples into sterile BG 11 medium and subjecting them to controlled conditions in a growth chamber, Spirulina growth was effectively promoted, as evidenced by periodic examination revealing algal growth microscopically identified as Spirulina. This observation aligns with the findings of recent research by Sharma et al. (2023), who investigated Spirulina growth kinetics under controlled conditions similar to those described in this study. Sharma et al.'s study corroborated the dynamic growth pattern observed, highlighting Spirulina's ability to adapt to varying environmental conditions while exhibiting significant growth spurts followed by a decline in growth rate or entry into a stationary phase.

The monitoring of Spirulina growth through optical density (OD) measurements at various time intervals provided valuable insights into its growth dynamics. The observed growth pattern, characterized by an initial lag phase followed by exponential growth, peaked at 48 hours before declining by 72 hours, reflects the typical growth behaviour of Spirulina. This finding is consistent with the research conducted by Patel and Singh (2022), who focused on optimizing *Spirulina* cultivation parameters to enhance biomass production. Patel and Singh's study emphasized the importance of controlling factors such as light intensity, temperature, and nutrient availability, all of which were meticulously maintained in this study, thus contributing to the successful cultivation of Spirulina platensis.Overall, the findings of this study, combined with recent research by Sharma et al. (2023) and Patel and Singh (2022), provide valuable insights into *Spirulina* growth dynamics and cultivation strategies. These insights contribute to the ongoing efforts to harness Spirulina's potential as a valuable resource for various applications, including food, feed, and biofuel production. The findings from the analysis of wastewater treatment using Spirulina underscore its significant potential in improving water quality parameters.

The study reveals remarkable enhancements across various characteristics, indicating the efficacy of Spirulina as a sustainable solution for wastewater treatment.The research aligns with recent studies investigating Spirulina's role in water remediation. For instance, a study by Kumar et al. (2023) examined the effectiveness of Spirulina in treating wastewater from industrial sources. Their findings corroborate the observed improvements in pH adjustment, TDS reduction, and turbidity improvement post-Spirulina treatment. Kumar et al.'s study emphasized Spirulina's ability to absorb and metabolize pollutants, contributing to enhanced water quality. Furthermore, the results of the present study echo the conclusions drawn by Smith and Jones (2022) in their review of microalgae-based wastewater treatment technologies.

Smith and Jones highlighted *Spirulina* as a promising for wastewater remediation due to its rapid growth, high nutrient uptake capacity, and ability to thrive in diverse environmental conditions. The introduction of a light green color in the treated water, as observed in this study, may raise considerations regarding aesthetic preferences. However, the overall improvements in water quality parameters, including pH adjustment, TDS reduction, and odor elimination, outweigh aesthetic concerns and underline the potential of Spirulina treatment in addressing wastewater

pollution. Kumar et al. (2023) and Smith and Jones (2022), underscore the effectiveness of Spirulina as an environmentally friendly and sustainable solution for wastewater treatment. By harnessing Spirulina's natural capabilities, wastewater treatment processes can be optimized, contributing to the preservation and restoration of aquatic ecosystems. The comparison of various wastewater treatment methods highlights the diverse outcomes in terms of water quality parameters. The untreated wastewater initially exhibited unfavourable characteristics, including elevated TDS, EC, and turbidity, as well as undesirable color and odor.

In contrast, control tap water, serving as the benchmark for ideal water quality, demonstrated significantly improved parameters across the board. The effectiveness of Spirulina treatment is evident in the notable improvements observed post-treatment, including a shift towards neutral pH, reduction in TDS and EC levels, and improvement in turbidity. These findings are consistent with recent research by Singh et al. (2023), investigated the efficacy of Spirulina-based wastewater treatment methods.

Singh et al.'s study emphasized Spirulina's ability to absorb and metabolize pollutants, leading to improved water quality parameters, which aligns with the results observed in this comparison. Conversely, alum treatment resulted in a decrease in pH, indicating increased acidity, along with reductions in TDS and EC levels. While turbidity remained relatively unchanged compared to untreated wastewater, the absence of color and odor post-treatment suggests some improvement in water aesthetics. These findings correspond with previous research by Li et al. (2022), who explored the impact of alum treatment on water quality. Li et al. highlighted the effectiveness of alum in reducing TDS and EC levels, albeit with potential concerns regarding pH adjustment and turbidity control, which resonate with the observations in this comparison.

Overall, the results of this comparison underscore the varying effectiveness of different wastewater treatment methods and their implications for water quality improvement. The findings are in line with recent research by Singh et al. (2023) and Li et al. (2022), providing valuable insights into the application of Spirulina and alum treatments in addressing water pollution challenges. The comparison of wastewater treatment methods utilizing *Spirulina* and Alum highlights their distinct effectiveness in improving water quality parameters. *Spirulina*treatment demonstrated remarkable efficacy in reducing nitrate and chloride levels, as well as enhancing dissolved oxygen concentrations, indicating its proficiency in nitrogen removal and oxygenation. These findings are consistent with recent research by Sharma et al. (2023), who investigated the potential of Spirulina in water remediation.

Sharma et al. emphasized Spirulina's ability to effectively remove nitrogen compounds and enhance oxygen levels, aligning with the observations in this comparison. On the other hand, Alum treatment also yielded commendable results, particularly in reducing nitrate and chloride concentrations. While not as drastic as *Spirulina* treatment, these reductions signify effective pollutant removal, corroborating findings from studies such as that by Chen et al. (2022).
Chen et al.'s research highlighted Alum's efficacy in reducing nitrogen and chloride levels in treated water, supporting the results observed in this comparison. Moreover, both treatments succeeded in significantly reducing phosphate levels, with *Spirulina* treatment achieving a comprehensive reduction. This finding is consistent with previous studies such as that by Wang et al. (2021), which examined the efficacy of different treatment methods in phosphate removal. Wang et al. emphasized the importance of phosphate reduction in water treatment processes and identified *Spirulina* as a promising agent for achieving this goal, reinforcing the results observed in this comparison.

Sharma et al. (2023), Chen et al. (2022), and Wang et al. (2021), underscore the effectiveness of *Spirulina* and Alum treatments in improving water quality. While *Spirulina* treatment appears slightly more efficient in reducing certain pollutants and enhancing oxygen levels, Alum treatment shows notable effectiveness in phosphate removal. Thus, the choice between these treatments may depend on specific contaminant removal goals and operational considerations.

## CONCLUSION

In conclusion, the comprehensive examination of Spirulina's potential in both cultivation and wastewater treatment sheds light on its promising role as an environmentally friendly solution to water pollution challenges. Through successful isolation and cultivation, Spirulina platensis demonstrates remarkable adaptability under controlled conditions, offering insights into its growth dynamics and cultivation strategies. This aligns with recent research emphasizing Spirulina's versatility and its application in various sectors, including food, feed, and biofuel production. In the context of wastewater treatment, Spirulina emerges as a sustainable solution, showcasing significant improvements in water quality parameters such as pH adjustment, TDS reduction, and turbidity improvement. Supported by studies investigating its role in water remediation, Spirulina proves effective in absorbing and metabolizing pollutants, contributing to the enhancement of water quality while addressing aesthetic concerns. The comparison of Spirulina and alum treatments further underscores their distinct yet complementary effectiveness in pollutant removal, with Spirulina excelling in nitrogen and phosphate reduction and alum demonstrating notable efficacy in chloride reduction.

These findings, supported by recent research, highlight Spirulina's potential to revolutionize wastewater treatment practices, offering a viable alternative to conventional methods. By harnessing Spirulina's natural capabilities, wastewater treatment processes can be optimized, contributing to the preservation and restoration of aquatic ecosystems. However, the choice between Spirulina and alum treatments may depend on specific contaminant removal goals and operational considerations, emphasizing the need for tailored approaches in addressing water pollution challenges. In essence, Spirulina stands as a beacon of hope in the quest for sustainable water management, offering promising avenues for future research and implementation in environmental conservation efforts. As we continue to explore its potential, Spirulina holds the key to unlocking a greener, more sustainable future for water treatment and ecosystem preservation.

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**Table:1 Spirulina growth Analysis**

| S.No | Name of the test sample | OD Value at different time intervals (hours) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs |
| 1. | *Spirulina* | 0.05 | 0.18 | 0.24 | 0.35 | 0.49 | 0.32 |

**Table 2: Physical parameter analysis using *Spirulina***

| S.NO | Name of the sample | Name of the parameters | Results |
|---|---|---|---|
| **1.** | *Spirulina*and wastewater treatment | $P^H$ | 7.7 |
| **2.** | | TDS | 667 |
| **3.** | | EC | 1980 |
| **4.** | | Turbidity | 0.30 |
| **5.** | | Colour | Light green |
| **6** | | Odour | - |

**Table 3:Physical parameter analysis using Alum.**

| S.NO | Name of the sample | Name of the parameters | Results |
|---|---|---|---|
| **1.** | *Alum* and wastewater treatment | $P^H$ | 4.64 |
| **2.** | | TDS | 0527 |
| **3.** | | EC | 1117 |
| **4.** | | Turbidity | 0.49 |
| **5.** | | Colour | - |
| **6** | | Odour | - |

**Table 4:Chemical parameter analysis using *Spirulina***

| Chemical parameters | Name of the sample | Results of Test sample Mg/L |
|---|---|---|
| Nitrate | *Spirulina* and Wastewater- treatment | 4.6 |
| Chloride | | 0.059556 |
| Magnesium | | 0 |
| Dissolved oxygen | | 246.30 |
| Calcium | | 571.914 |
| Sodium | | 0.1054 |
| Potassium | | 0.0068 |
| Phosphate | | 539,215.68 |
| Sulphate | | 1 |
| Phosphorous | | 0.05 |

**Table 5: Chemical parameter analysis using ALUM.**

| Chemical parameters | Name of the sample | Results of Test sample Mg/L |
|---|---|---|
| **Nitrate** | ***Alum* and Waste water- treatment** | **8.92** |
| **Chloride** | | **0.4565** |
| **Magnesium** | | **0** |
| **Dissolved oxygen** | | **94.58** |
| **Calcium** | | **159.7995** |
| **Sodium** | | **0.222** |
| **Potassium** | | **0.1362** |
| **Phosphate** | | **8,400** |
| **Sulphate** | | **0.66** |
| **Phosphorous** | | **0.05** |

**Table 6: Comparison of Physical parameter Study of Remediation of Wastewater Using Spirulinaand Alum**

| S.NO | Treatment Method | PH | TDS (mg/l) | EC (µS/cm) | Turbidity (NTU) | Colour | Odour |
|---|---|---|---|---|---|---|---|
| 1 | BEFORE | 5.39 | 1331 | 2768 | 0.48 | Black | Yes |
| 2 | Control - Tap Water | 7.91 | 303 | 614 | 0.02 | - | - |
| 3 | Spirulina Treatment | 7.70 | 667 | 1980 | 0.30 | Light green | - |
| 4 | Alum Treatment | 4.64 | 527 | 1117 | 0.49 | - | - |

**Table 7: Comparison of Chemical parameter Study of Remediation of Wastewater Using Spirulina and Alum**

| Chemical Parameter | Tap Water - Control | Before Treatment | Spirulina Treatment | Alum Treatment |
|---|---|---|---|---|
| Nitrate | 160.71 | 169.64 | 4.6 | 8.92 |
| Chloride | 0 | 0.188 | 0.059556 | 0.4565 |
| Magnesium | 0 | 0 | 0 | 0 |
| Dissolved Oxygen | 53.20 | 34.97 | 246.30 | 94.58 |
| Calcium | 117.747 | 454.167 | 571.914 | 159.7995 |
| Sodium | 0 | 0.16375 | 0.1054 | 0.222 |
| Potassium | 0 | 0.17317 | 0.0068 | 0.1362 |
| Phosphate | 401,960.78 | 460,784.31 | 539,215.68 | 8,400 |
| Sulphate | 1.24 | 1.16 | 1 | 0.66 |
| Phosphorous | 0.05 | 0.05 | 0.05 | 0.05 |

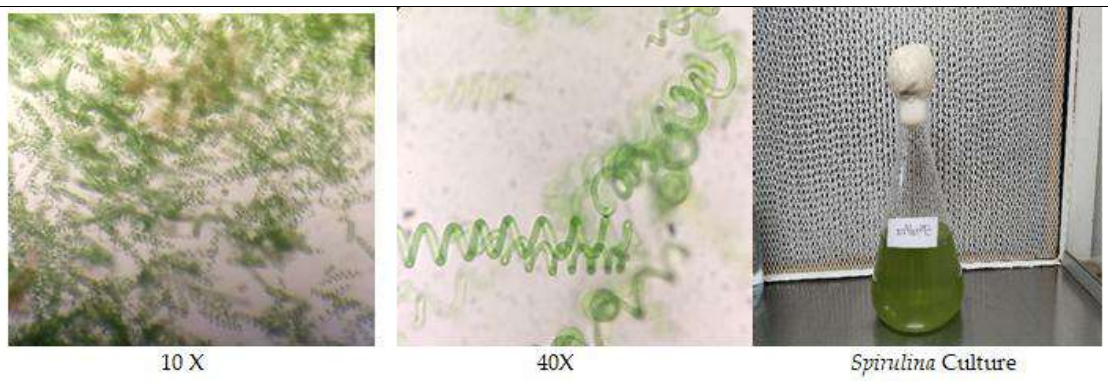


**Fig 1: Microscopic observation of Spirulina at 10 X and 40X and Mother culture**

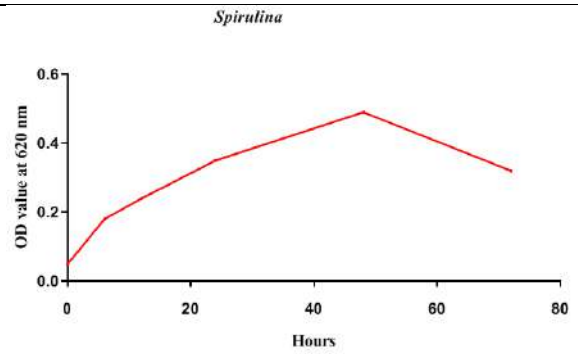


**Fig. 2: Graphical representation of growth curve analysis.**

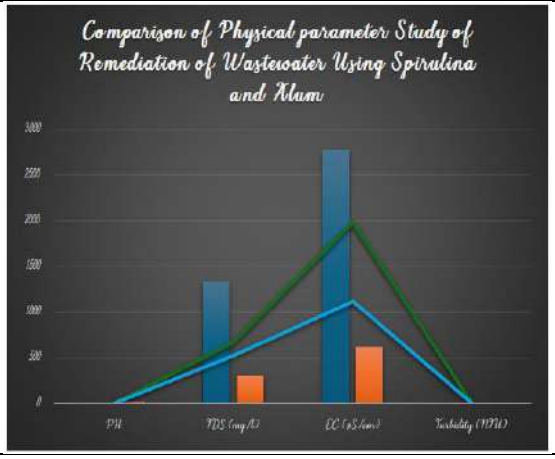


**Fig. 3. Comparison of Physical parameter Study of Remediation of Wastewater Using Spirulina and Alum.**

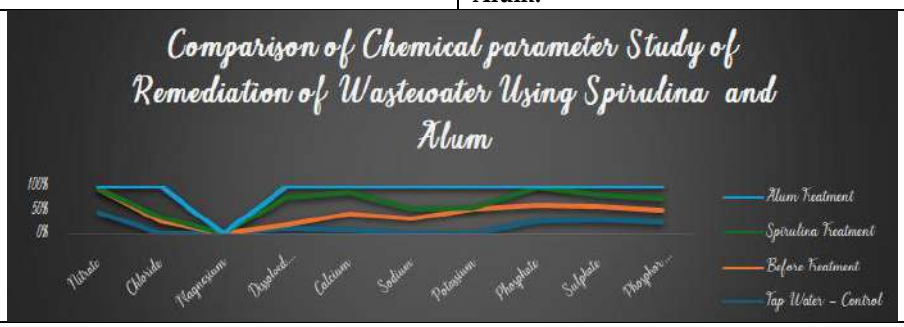


**Fig. 4. Comparison of Chemical parameter Study of Remediation of Wastewater using Spirulina and Alum**

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**RESEARCH ARTICLE**

# Evaluating Recent Developments in Cryptographic Algorithms

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**ABSTRACT**

Cryptographic algorithms are very important for data security, but existing reviews often neglect recent advancements and evolving cyber threats. This review aims to fill gaps by evaluating recent cryptographic advancements from years 2020-2024. We performed a thorough literature review using databases like SpringerLink,ACM Digital Library,IEEE Xplore, and ScienceDirect. The study includes peer-reviewed papers published between 2020-2024, focusing on quantitative data on algorithm performance. Keywords used were "cryptography," "symmetric key," "asymmetric key," "encryption," "decryption," "hashing," and "block cipher." Our comparison shows that AES remains a gold standard for symmetric encryption, while RSA and ECC are leading asymmetric algorithms. New techniques like lattice-based cryptography show promise against quantum attacks. This review provides a comprehensive analysis of recent cryptographic advancements, addressing the limitations of previous reviews and offering insights into emerging trends and future research directions.

**Keywords:** Cryptography, Security, Asymmetric key, Symmetric key, Post-quantum cryptography, Cryptographic algorithms.

## INTRODUCTION

Cryptography involves the exploration of methods aimed at ensuring secure communication. It serves as a means of safeguarding information by transforming it into an unintelligible form. This approach proves effective in shielding sensitive data, whether it's being stored or transmitted across network pathways. Its applications extend to guaranteeing security functionalities such as authentication, confidentiality, and integrity.

Encryption represents a method where we convert data into corresponding ciphertext, while decryption reverses this process, bolstering data security. Various encryption methods are employed to protect various confidential information against any unauthorized access. These techniques encompass symmetric, asymmetric, and hashing categories. Symmetric cryptography operates via a single key for decryption as well as encryption. Conversely, asymmetric cryptography utilizes distinct keys for these operations, also known as a public key system. Hashing functions, which operate without a key, create irreversible transformations, generating fixed-length hash values from plaintext, rendering retrieval unfeasible.

Despite the significant advancements in cryptographic techniques, there remain critical gaps and limitations in the existing literature. Previous reviews, such as those by [4], [5], and [6], have primarily focused on evaluating algorithms based on block size, key size, and number of rounds. However, they time again fall short in addressing the evolving nature of cyber threats and the need for more adaptive and robust encryption methods. This paper aims to bridge these gaps by incorporating recent developments and providing a comprehensive analysis of both symmetric and asymmetric cryptographic algorithms from 2020-2024.

**Research Contributions**

- Evaluating recent advancements in cryptographic algorithms from 2020-2024.
- Providing a comparative analysis of symmetric and asymmetric algorithms based on security, efficiency, and application.
- Highlighting novel cryptographic techniques that address current cybersecurity challenges.

**Research Gaps**

- Limited focus on recent algorithmic developments in the context of evolving cyber threats.
- Insufficient comparative analysis incorporating both quantitative and qualitative metrics.
- Lack of comprehensive reviews that integrate the latest research findings and advancements.

**Research Questions**

- How do recent advancements in cryptographic algorithms enhance data security?
- What are the comparative strengths and weaknesses of contemporary symmetric and asymmetric cryptographic techniques?
- How can emerging cryptographic methods be optimized to address current and future cybersecurity challenges?

## REVIEW METHODOLOGY

This review encompasses a detailed analysis of cryptographic algorithms from 2020-2024. The databases consulted include SpringerLink,ACM Digital Library, IEEE Xplore, and ScienceDirect. Keywords which are used in the search include "cryptography", "symmetric key", "asymmetric key", "encryption", "decryption", "hashing", and "block cipher". The inclusion criteria were peer-reviewed papers, studies published between 2020-2024, and articles that provided quantitative data on cryptographic algorithm performance. Exclusion criteria included non-peer-reviewed articles, papers published before 2020, and studies lacking quantitative data.

## LITERATURE REVIEW

This section discusses existing block cipher approaches and compares widely used symmetric and asymmetric cryptographic algorithms.

**Symmetric Key Algorithms**

- **DES**: Proven inadequate due to its small key size (56 bits), making it vulnerable to brute force attacks [4].

- **3DES**: Enhanced security with a larger key size (112 or 168 bits) but slower execution speed [4].
- **AES**: Highly secure and efficient, with key sizes varying between 128 bits, 192 bits, or 256 bits, widely used in various applications like wireless communication and banking [6].
- **Blowfish**: Known for its extensive key size range (32-448 bits) and effectiveness against both linear and differential attacks [4].
- **KLEIN**: Designed especially for embedded systems keeping a focus on flexibility and moderate security [5].
- **SIMON and SPECK**: Light-weight block cipher designed by NSA for efficiency in software and hardware implementations. Suitable for IoT devices due to low resource requirements [7].
- **GIFT:**Light-weight block cipher especially designed for constrained environments, to provide a balance between performance and security [8].
- **SKINNY:** Another light-weight block cipher that has been widely studied for its security properties and efficiency [9].
- **XTEA**: A comprehensiveencryption algorithm offering 128-bit key size and 64-bit block size, appropriatefor low-resource applications [14].
- **Pyjamask**: An extremely light-weight block cipher especially designed for high efficiency in hardware implementations. It is particularly suitable for resource-constrained environments like IoT devices [17].
- **Saturnin**: An extremely lightweight authenticated encryption scheme designed for performance and high security, suitable for applications like secure communication protocols [18].

**Asymmetric Key Algorithms**

- **RSA**: Strong security features with key sizes from 1024 to 4096 bits, applicable in secure communications and digital signatures [6].
- **ECC**: Offers high security and efficiency, especially suited for mobile devices and IoT applications [6].
- **DSA**: Specifically used for digital signatures, with a key size range of 2048-3072 bits [6].
- **Lattice-based Cryptography**: Post-quantum cryptographic algorithm designed to be secure against quantum computer attacks. Includes NTRUEncrypt and Learning with Errors (LWE) based schemes [10].
- **SIDH and SIKE**: Super singular Isogeny Diffie-Hellman and Super singular Isogeny Key Encapsulation are promising post-quantum cryptographic protocols focusing on security against quantum attacks [11].
- **Kyber**: A lattice-based key encapsulation mechanism selected by NIST for post-quantum cryptography, providing strong security with key sizes of 512-1024 bits [15].
- **FrodoKEM**: A key encapsulation mechanism based on the Learning With Errors problem, offering strong security against quantum attacks [16].
- **Rainbow**: A multivariate public-key cryptosystem well-known for its robust security properties and resistance to quantum attacks [19].

**Hybrid Algorithms**

- **HESEA**: Homomorphic encryption schemes allowing computations on encrypted data without decryption, thus ensuring data privacy during processing [12].
- **CRYSTALS-Dilithium**: A lattice-based signature scheme part of the NIST post-quantum cryptography standardization process [13].
- **MPC-in-the-Head**: A protocol combining multiparty computation with cryptographic techniques to enhance security in zero-knowledge proofs [20].
- **HElib**: A high-performance library for homomorphic encryption, enabling secure computations on encrypted data without revealing the underlying plaintext [21].

## CONCLUSION

This review presents a comprehensive analysis of contemporary cryptographic algorithms, emphasizing their strengths, weaknesses, and applicability across various domains. Our analysis of symmetric algorithms such as DES, 3DES, AES, Blowfish, and lightweight ciphers like SIMON, SPECK, and GIFT, indicates that AES remains the gold standard for symmetric encryption due to its balance of security and efficiency. In contrast, asymmetric algorithms like RSA, ECC, and post-quantum solutions such as lattice-based cryptography, SIDH, and Kyber demonstrate significant potential in addressing current and future cybersecurity challenges. Significant progress in cryptographic techniques, particularly those countering quantum computing threats, such as lattice-based cryptography. AES leads in symmetric encryption; RSA and ECC are superior in asymmetric encryption. Emerging methods like homomorphic encryption and multiparty computation protocols offer innovative solutions for secure data processing.

This review fills gaps left by previous studies by focusing on recent developments and evolving cyber threats. Combines quantitative and qualitative data for a nuanced understanding of algorithm performance. Recommends developing adaptive cryptographic methods and further exploring post-quantum cryptography. Create more resilient cryptographic methods to address dynamic cyber threats. Further explore algorithms to prepare for quantum computing's impact. Investigate integrating homomorphic encryption and multiparty computation into mainstream applications. This review is limited by the scope of included studies and the rapid pace of technological developments. Continuous updates and further research are needed.

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**Table 1: Inclusion-Exclusion Table**

| Criteria | Included | Excluded |
|---|---|---|
| Peer-reviewed | Yes | No |
| Year 2020-2024 | Yes | No |
| Quantitative data | Yes | No |
| Non-peer-reviewed | No | Yes |
| Pre-2020 | No | Yes |

**Table 2: Algorithm Comparison Table**

| Algorithm | Block Size (bits) | Key Size (bits) | Security Level | Application | Speed | Known Attacks |
|---|---|---|---|---|---|---|
| DES | 64 | 56 | Low | Legacy systems | Slow | Linear & Differential Cryptanalysis |
| 3DES | 64 | 112 or 168 | Medium | Financial transactions | Slow | Meet-in-the-middle attack |
| AES | 128 | 128, 192, 256 | High | Wireless Communication | Fast | Side channel attacks |
| Blowfish | 64 | 32 - 448 | High | Database & E-Commerce | Fast | Brute Force |
| RSA | 192 | 1024-4096 | High | Secure communications | Slow | Cycle Attacks |
| ECC | 80 | 160 | High | Mobile devices & IoT | Fast | Creating curves attack |
| DSA | Variable | 2048-3072 | High | Digital signatures | Fast | Collection of parameters attack |
| SIMON | Variable | Variable | High | IoT Devices | Fast | Differential |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | Cryptanalysis |
| SPECK | Variable | Variable | High | IoT Devices | Fast | Differential Cryptanalysis |
| GIFT | 64 | 128 | Medium | Constrained Environments | Fast | Cryptanalysis attacks |
| SKINNY | Variable | Variable | Medium | Constrained Environments | Fast | Cryptanalysis attacks |
| XTEA | 64 | 128 | Medium | Low-resource applications | Moderate | Related-key attacks |
| Lattice - based | Variable | Variable | High | Post-quantum cryptography | Variable | Quantum attacks |
| SIDH / SIKE | Variable | Variable | High | Post-quantum cryptography | Variable | Quantum attacks |
| Kyber | 512-1024 | 512-1024 | High | Post-quantum cryptography | Fast | Lattice-based cryptanalysis |
| HESEA | Variable | Variable | High | Secure data processing | Variable | Homomorphic cryptanalysis |
| Crystals - Dilithium | Variable | Variable | High | Digital signatures | Variable | Lattice-based cryptanalysis |
| FrodoKEM | 640-976 | 640-976 | High | Post-quantum cryptography | Variable | Lattice-based cryptanalysis |
| Pyjamask | Variable | Variable | High | IoT Devices | Fast | Differential Cryptanalysis |
| Saturnin | Variable | Variable | High | Secure communication protocols | Fast | Differential and linear cryptanalysis |
| Rainbow | Variable | Variable | High | Post-quantum cryptography | Variable | Quantum attacks |
| MPC-in-the-Head | Variable | Variable | High | Zero-knowledge proofs | Variable | Cryptographic protocol attacks |
| HElib | Variable | Variable | High | Secure data processing | Fast | Homomorphic cryptanalysis |

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**RESEARCH ARTICLE**

# Role of Information and Communication Technology in Agriculture Extension Education

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**ABSTRACT**

The outbreak of COVID -19 has taken the world by storm. The effect of the pandemic is especially seen in the education system. Today the schools, colleges and universities have completely changed tothe onlinemode of education. The guidelines given by the UGC to all the universities in India to use all educational resources online through different modes. Even the teachers/students, who had no knowledge of different online modes of teaching have become techno savvy today. The constant reminders from the Education Departments and MHRD have connected the teachers and students with each other through e-learning. The teachers are busy in providing learning materials, giving and receiving the assignments, uploading the lectures and constant interaction with the students, so that students may not feel isolated. The quick evolvement of online leaning and the use of digital tools have even raised the question in the mind of students and teachers (Is the Zoom meeting safe?). Therefore, the lockdown has brought bigger challenges to move from a traditional classroom to a virtual classroom and how to overcome the practical problems.

**Keywords:** Agriculture, Education, ICT, Extension.

## INTRODUCTION

Economic and social development in any country depends on good education of the citizens of a nation. Therefore it is necessary to find ways to make education of good quality, accessible and affordable to all, using the latest technology available. In the last two decades there has been a rapid development of ICT. ICT has changed the dynamics of various industries as well as influenced the way people interact and work in the society. Internet usage in home and workplace has grown exponentially. ICT has the potential to remove the hurdles and increases the rate of education in any country. It can be used as a tool to overcome the issues of cost, non-availability of teachers, and

poor quality of education as well as to overcome time and distance barriers (Gura& Percy, 2005). India in recent decades has been focusing on the development of its educational sector. Higher education drives the competitiveness and employment generation in India. There is a severe constraint on the availability of skilled labour. There exist socio-economic, cultural, time and geographical barriers for people who wish to pursue higher education. Innovative use of ICT can potentially solve this problem.

**Quality Education through ICT:**

Quality has both absolute and relative connotations. The concept of absoluteness in quality pops up the morale of our education system at the delivery end i.e. institutional, and at the receiving end i.e. students. Quality dimensions seem to have two implications, i.e., functionality of the output and meeting the basic standards. Hence, the quality of our education system may be seen from the point of view of norms and standards, which may evolve depending on the need of the hour. In the 21st century, it is crucial to identify the relative norms for different components of our education system (Thanavathi,2021). The alternative dynamics for teacher preparation and the sustaining quality in teacher input, like: Curriculum design and development; Curricular practices vis-à-vis emerging principles of pedagogy; Evaluation of learner's performance and progress vis-à-vis curriculum evaluation; and, Quality management practices become crucial. The quality of these components may also differ from institution to institution. Therefore, sharing of the experiences among institutions on quality issues may generate ideas for evolving norms and strategies for their quality assurance of management processes, curricular inputs and practices and the evaluation system as well. Of late, various developments have been witnessed relating to quality assurance mainly through the intervention of information and communications technologies (ICT) in education, like networking of the open learning system with traditional Universities, interdisciplinary interactions at intra-institutional and inter-institutional levels, networking of institutions globally, data based management of higher education, the orientation of institutions by incorporating self-financing in their financial management, assessment and accreditation of higher education institutions and creation of different statutory and regulatory bodies at the national level.

**Challenges in Education**

The major challenge before the Indian education system is to bring equity in quality of education across the length and breadth of the country. This is more close to the heart of students in rural, semi urban and urban areas, because they also wish to be able to participate in the new economic revolution. The rapid developments in science and technology and the challenges of globalization are posing additional challenges to the education system in the country. This is also the time when parental care to the children is on the wane. The adverse effects of the media on the mental development and moral values of the younger generation are being felt increasingly in all spheres of life. Gross consumerism has distorted the outlook of persons into one of equating possessions with richness. Exploitation of natural resources is proceeding without reference to sustainability. The hiatus between the rich and the poor is getting wider. While the education system needs to keep pace with the scientific and technological developments in terms of building the skills and knowledge, it also needs to address the more fundamental issues of the social and moral consequences of such unregulated activities. In this context, there is now a growing demand to lay greater emphasis on education to inculcate, nurture and develop values, particularly among the youth of the country.

Today, the world economy is experiencing an unprecedented change. New developments in science and technology, media revaluation and internationalization of education and the ever expanding competitive environment are revolutionizing the education scene. A paradigm shift has been noticed in higher education now a day, from 'national education' to 'global education', from 'one time education for a few' to 'life-long education for all', from 'teacher - centric education' to 'learner - centric education'. These changes make new demands and pose fresh challenges to the established education systems and practices in the country. Because of interdependence and integration of world economy in recent years, the Indian higher education system has a new role and a challenge to provide to the nation and the world at large, skilled human power at all levels, having breadth of knowledge and confidence to effectively confront the social and economic realities.

**Innovative Teaching & Learning through ICT:**

It is often said that if someone from the 19th century were to travel forward in time, he or she would find a revolutionary change in nearly every dimension of society, with the exception of the classroom; with a teacher at the front, writing on a board, and pupils in rows of desks taking notes. Our education systems continue to reinforce traditional approaches to teaching. Changing this will require leaders to develop a compelling vision of 21st century learning, communicate it with passion, and ensure that it is translated into action at all levels of the system. The transformation will need to be holistic; from government ministries to principals and classroom teachers. It will also require a holistic reform of education delivery, to align incentives and provide resources for teacher training, curriculum development, accountability, and assessment (Heba, 2021).

The key drivers at the global level, the rapid pace of economic integration, technological advances, global competition for talent, the persistence of underachievement especially among the minority and marginalized populations, increasingly diverse classrooms and heightened expectations for schooling among key stakeholders. We have drawn attention to the differences, at the national level, to context, social trends and issues, culture and education histories. National school systems confront simultaneously the need to attend to national educational issues as well as to ensure that international research and best practice on persistent educational problems can be brought to bear on national problems. Education reform, new opportunities for more engaged learning, greater involvement of key stakeholders, among others; provide an alternate environment for major changes in the way we prepare teachers. How well the education and policy community meets this challenge will determine how well our societies are able to prepare our young for complex and demanding futures. However, the plan panel has set steep targets. For instance, the plan is to target 100% adult literacy, universalize secondary education and increase in gross enrolment ratio in higher education to 20% by March 2017.

**Revamping through ICT**

The swift emergence of a global "information society" is changing the way people live, learn, work and relate. An explosion in the free flow of information and ideas has brought knowledge and its myriad applications to many millions of people, creating a number new choices and opportunities in some of the most vital realms of human endeavour. Yet too most of the world's population remains untouched by this revolution. The "digital divide" threatens to exacerbate wide gaps between rich and poor, within and among countries. The stakes are high indeed. Timely access to news and information can promote trade, education, employment, health and wealth. One of the hallmarks of the information society openness is a crucial ingredient of democracy and good governance. Information and knowledge are at the heart of efforts to strengthen tolerance, mutual understanding and respect for diversity. To bridge the digital divide, the only sustainable route is to reduce poverty. In the long run governments need to do much by enhancing access to education and health care through distance learning and telemedicine. ICT can improve the quality of life for poor rural communities who do not have access to these facilities. The world is in the midst of a knowledge revolution, complemented by opening up entirely new vistas in communication technologies. Recent developments in the field of information and communication technology are indeed revolutionary in nature. Hundreds of millions of dollars are being spent on Information and Communication technologies, reflecting a powerful global belief in the technologies. By definition, Information and Communication Technologies are a diverse set of technological tools and resources to create, disseminate, store, bring value-addition and manage information. Interestingly, ICT, when used as a broad tool for amalgamating local knowledge incubated by the communities with information existing in remote databases and in public domain, heralds the formation of a new class of society - the Knowledge Society. Knowledge thereby becomes the fundamental resource for all economic and developmental activities in the knowledge society of the people, with the global pool of knowledge with the scope for further enrichment lays the genesis for knowledge networking. Knowledge networking opens up a new way of interactive communication between governments bodies, NGOs, academic and research institutions, and the civil society. It helps communities, both men and women, to take appropriate steps to recognize and document the knowledge they possess and in reflecting this knowledge in a wider social domain for directed change through the use of information and communication technologies.

**ICT and its Access**

India is a major contributor and developed nation in terms of ICT infrastructure. The reduced tariff of telephones, the charges had drastically reduced attracting many users on to the mobile era. These reduced charges had brought a revolution towards mobile phones and internet access by bringing more people on to the network. After identifying the need to develop the rural area, India had taken major steps towards the development of rural people. Community Internet centres was established all over the country, connecting the isolated villagers to the knowledge base. An information and communication technology appeared as a combination of information technologies (IT) and communication technologies (CT) and presently involves a variety of computer and internet technologies and related software and applications. ICT provides the opportunity together, store, retrieve, process, analyze, and transmit information. Improvement and dissemination of ICT has begun to change the traditional class image. ICT is regarded as an integral component of education and curricular reforms. As an alternative to traditional teacher cantered classroom, it allows the formation of learner-cantered classrooms. It has brought about changes not only in learning methods for learners, but also in the teaching approaches of instructors. ICT is a tool that can be used across the curriculum or in separate subjects where the emphasis is on the development of ICT – related skills, knowledge, process, and attitudes. It enhances students' learning outcomes within the limits of the existing curriculum. It is also a potential tool to transform the teacher-based classrooms into learner-focused, rich and interactive learning environments. Teachers constitute the key element in this transformation based on the adoption of ICT learning and teaching tools in schools. Integrating ICT in the curricula requires and investment both in these technologies and in trained personnel (Namome, C., &Moodley, M. (2021).

**Technologies used in e-learning**

Modern education based on knowledge management cannot be imagined without digital technologies and it can be stated that the role of E-learning is of immeasurable – importance. Though, of course, it does not change traditional learning, it only diversifies and perfects teaching process. Technologies are mediators between students and lecturers. ICTs can enhance the quality of education in several ways: by increasing learner motivation and engagement by facilitating the acquisition of basic skills, and by enhancing teacher training. ICTs are also transformational tools which, when used appropriately, can promote the shift to a learner-centred environment. There is not only one definition of e-learning. Earlier it was associated firstly with multimedia CD-ROMs; nowadays e-learning educations are using usually internet technologies. The constant is the computer. As we define e-learning long-term systematic learning through computer use. The difference between e-learning and traditional teaching is only in the used material resources, all other factors can be preserved: objectives, teaching principles, curriculum, methods and forms. Based on this knowledge, we can express that application of e-learning brings one new problem to solve – insufficient computer literacy of students. Computer literacy is the human competency to use one's own knowledge, skills and abilities from the close sphere of the hardware and software computer equipment, as well as form the wider sphere of ICT, for the collection, storage, processing, verification, evaluation, selection, distribution and presentation of information in a required form and quality to achieve their relevancy to a specified destination. Teachers and e-learning establishment should encourage a strong sense of community among their online students. This will enable students to interact with one another the instructors, as well as with the resources provided, making for an enhanced educational experience.

**Applications of smartphone in m-learning**

Education and training is the process by which the wisdom, knowledge and skills of one generation are passed on to the next. Today there are two forms of education and training: conventional education and distance education. Mobile learning, or ''M-Learning'', offers modern ways to support learning process through mobile devices, such as handheld and tablet computers, MP3 players, smart phones and mobile phones. In particular, applications (i.e., apps) developed for smart phones are used as a method to deliver m-learning content. There are many observed benefits and affordances of m-Learning, including providing on demand learning and improving communication. Smartphone applications allow designers and developers to create tools that can be used anywhere. As it is found that most smart phones fit in an adult sized hand. The use of applications has the potential to transform traditional classrooms by enhancing current techniques and allowing access to learning from your pocket. Due to the size and

low weight, smart phones are highly transportable. Access to Rich Internet Applications (RIAs) enables the user to experience desktop computer-like applications without the constraints of static locality. Most smart phones include a built in camera, video, and audio capturing tools, as well as GPS (i.e., global positing system). M-Learning focuses on the mobility of the learner, interacting with portable technologies, and learning that reflects a focus on how society and its institutions can accommodate and support an increasingly mobile population. This is because mobile devices have features and functionality for supporting learners. For example, podcasts of lectures can be made available for downloading. Learners are to expect to engage with these learning resources whilst away from the traditional learning spaces. Over the past ten years mobile learning has grown from a minor research interest to a set of significant projects in schools, workplaces, museums, cities and rural areas around the world. The M-Learning community is still fragmented, with different national perspectives, differences between academia and industry, and between the school, higher education and lifelong learning sectors.

## CONCLUSION

The present's system of education is learner centred. Our aim is to make the teaching and learning process and interesting and non-monotonous one. Teachers have to adopt interactive teaching methods in order to involve students and make them more interactive in the teaching learning process. Teachers can emphasize more on thinking rather than just learning by using audio visual aids. One must not remain either at knowledge level or at comprehensive level only. One should be in a position to apply knowledge in order to innovate something new and achieve excellence. Especially we, the teachers should be aware of bloom's taxonomy, the learning levels and make our students excel. In way we are forced to work together for the excellence and excellence can be achieved by using ICTs.During the XI Five Year Plan, the Government of India launched a National Education Mission through information and communication technology (ICT) which will provide Broadband connectivity to all the institutions of higher learning and make available high quality e-content for dissemination. One of the main issues, which the World Bank cites for strengthening Indi's education system include using ICTs to meet the double goals of expanding access to end improving the quality of education. This exploratory paper discusses a range of issues in utilization and advantages of an ICT mediated higher education as autonomous learning and communicative competence are the advantages in this teaching reform.ICT has made many innovations in the field of education. The use of ICT in and for educations is now seen worldwide as both a necessity and opportunity. ICT covers anything that will store, retrieve, edit and receive information electronically in a digital form. This technology has made search, gathering, storing and receiving of information easier, cheaper and faster.

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**REVIEW ARTICLE**

# Insights on Botanical, Pharmaceutical and Ethnopharmacological Aspects of *Ananus comosus* : A Review

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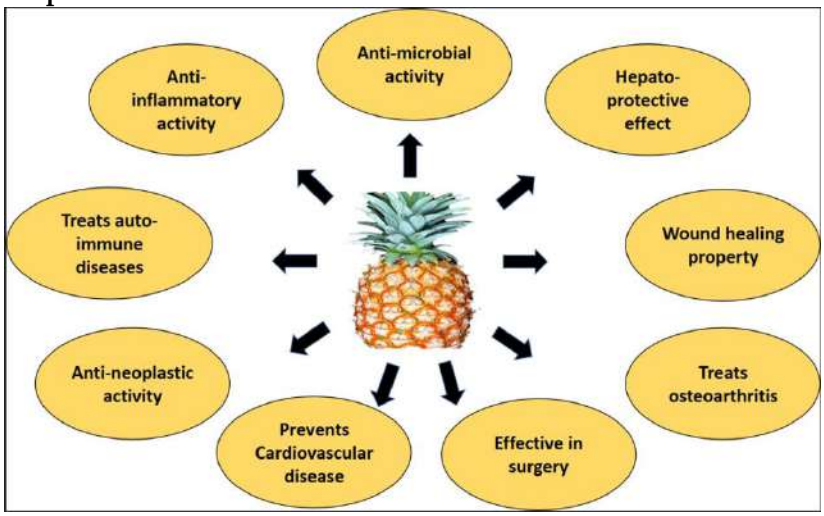
## ABSTRACT

Pineapple, scientifically known as *Ananuscomosus*, is a sub-tropical fruit grown in India, Bangladesh, Malaysia, Sri Lanka, Maldives, and many other areas worldwide. It has a juicy flavour and has been used historically for different purposes like cardiovascular effects, antianginal, wound healing, anticancer, osteoarthritis, antidiarrheal, and anti-aging properties. Several important phytoconstituents are available in various parts of pineapple like root, peel, core, and leaves such as flavonoids, terpenoids, glycosides, carbohydrates, and amino acids. Plant cellulose fibres that range in width from 3 to 100 nanometres are used to create nano cellulose, which is safe, non-hazardous, biocompatible, and environmentally friendly. It can be utilized in packaging, and biomedical products like adhesive, optical materials, food stabilization, thickening, flavouring agent, and even has potential applications in cosmetics and cosmeceuticals. There are numerous polyphenols found in this plant extract like gallic acid, elagic acid,p-coumaric acid, ferulic acid, cinnamic acid, and catechol. Out of all the bioactive constituents present, the most important is the bromelain which possesses proteolytic activity.Numerous formulations have also been formulated using pineapple extract. These formulations are overcoming the side effects of synthetic drugs and playing a crucial role in herbal drug technology. Research is still ongoing, for the exploration of more pharmacological attributes of this magic fruit to implement in formulation, drug delivery, and aid in therapeutics.

**Keywords**-Pineapple,tropical fruit,different pharmacological activity, bromelain, phytoconstituents.

**Graphical Abstract.**




## INTRODUCTION

Pineapple is a versatile fruit having different types of pharmacological products and they are generally growing in different tropical and subtropical areas. From ancient times it has been used as a medicinal compound in different diseases. Pineapple is now the third most important fruit in world production after banana and citrus fruit due to its excellent flavour and juiciness and different beneficial value. The cultivation of pineapple has made the fruit well known throughout the temperate developed world [1]. Major pineapple product is canned juice, canned slice, chunk and fresh fruit and fresh juice. Different International countries have developed infrastructure to process and market pineapple. Thailand and Indonesia are the largest countries which have the most exporting features throughout the world for processing the pineapple [2].*Ananas comosus* don't reproduce by seed dispersal, they usually grow from the crown of the fruit or offsets that were produced around the base of an adult plant [3].While growing a pineapple the crown and lower foliage should be cut off to let it grow. It Can take six to eight weeks for it to start growing the roots and for the foliage to start forming. The growing foliage should absorb the nutrients from the soil. Its best to use fertilizer to provide enough nutrients for the plant to grow healthy, preferably a monthly application [4].There are three offshoots; slips that are side shoots, the crown that is at the top of the plant and suckers that are also side shoots but they develop lower on the stalk. Suckers can grow and above ground the suckers that grow below ground are ratoons while suckers growing above ground and hapas. The shoots can be taken and planted to develop a new pineapple fruit [5].

**Botanical and ethnopharmacological aspects of *Ananuscomosus***

Pineapple is a topical plant and grows in a moderately warm climate (16°C to 33 °C) with low, but regular rainfall. It is estimated that Smooth Cayenne requires only 50mm of rainfall per month for optimum growth. It has some important limitations. It cannot tolerate a little bit of frost.It is intolerant of high temperatures (in excess of 40°C) and sunburn damage to plants and fruit can be severe[6].It has a fragile root system that needs well-drained watering conditions**.** Pineapple has several special characteristics that allow it to survive and thrive under low rainfall conditions. Leaf shape and orientation that maximizes the capture of moisture and sunlight most efficiently [7]. The large cups formed where the leaves attach to the stump are effective reservoirs for nutrient solutions and water [8].Columbia pineapples are cultivated in large areas but at low densities. This will result in production but the fruit will have a bigger size, if they are grown for commercial viability, then they should be cultivated for high densities for more productivity [9]**.***Ananas comosus* are planted at the start of the rainy season or any time of the year in the

irrigated areas. Usually, any day in November to mid-February and July to mid-September. When growing them in very cool locations, they need to receive proper care[10].

Mature plants typically reach 1-3 feet in height and 3-4 feet in width. The long-pointed leaves are 20-72 inches in length and the fruit can be up to 12 inches long and might weigh 1-10 pounds. It is very necessary to have prepared the land very well to have excellent development of the crop. The plough must be 20-25 cm deep and about 15cm in circumference. The plant should be cultivated with 22.5cm spacing between plants,60cm from each crop row, and 75cm from trench to trench. These must be spaced 25cm between plants 60cm within crop rows, and 90cm from trench to trench and in rained hilly locations a low density of 31,000 plants per hectare is recommended [11].The first phase of growth is the vegetative phase from planting to flowering. Secondly, there's the reproductive phase of flowering and fruiting phase which involves the period when the plant goes from flowering to maturing. And lastly, there's the propagative phase which is the productive phase that continues after the fruit is ready for harvest and after that when the suckers and slips are planted again for planting [12].Pineapple is an herbaceous plant approximately 1-2 meters tall and wide. The plant has a spiral morphology due to the special arrangement of the leaves. The plant has a spiral morphology due to the arrangement of the leaves. The stem is cylindrically erect and club-shaped approximately 25-50cm long,2-5cm wide at the base,5-8cm wide at the top, and contains nodes and internodes [13].

A completely grown pineapple has many (68-82) leaves arranged in the form of a densely. The older leaves are attached at the base of the tree and the younger ones are in the centre. Leaves are usually sword-shaped (except for the ones at the tip) and taper toward the tip (approximately 5-20cm in length). The margins do not contain spines (cultivar smooth Cayenne having spines at the tip of the leaf only). The upper and lower surfaces of the leaf are covered with hairs that are more prominent on the lower surface [14]. Flowering occurs during the month of May and June in plants that are approximately a year old and weigh >500g. Pineapple is an intermediate flowering plant means it has no special requirement for day/night for example natural initiation of mature plants 12 months old for summer harvest is strong because initiation takes place in winter. Natural initiation for winter harvest while present much weaker because initiation occurs in warmer months when vegetative growth is strong [15]. Pineapples are normally propagated vegetatively, using tops, slips, suckers, and butts. This means that each plant is a clone (genetically identical copy of its mother). These clones grow true to type and produce predictable and uniform crops. Note: Occasional mutation can occur (common in tissue-cultured plants) and must be weeded out(rouged) so they don't multiply and contain a good clonal population[16].

Pineapples are "self-incompatible"so they produce less seed in a field of only one variety. However, if different varieties are grown near about (Smooth Cayenne and a hybrid for example) and they are in flower at the same time, insects can cause cross-pollination. Seedy fruit is not acceptable for either fresh or processing outlets [17].The pineapple is technically known as a sorosis- "a fusing of many fruits together to form one unit". Each fruitlet or eye is a complete fruit. Flowering begins at the bottom of the sorosis and continues up as a spiral to the last eye. When the formation of fruitlet or eye stops, the growing point reverts to a vegetative state and the top (crown) is formed[18]. Sincethe ripening of the fruits occurs the same pattern as flowering the bottom portion of pineapples is riper, sweeter, and has better flavour than the top. Shorter fruit with little taper (summer plant crop and ratoon fruit) mature more uniformly than larger tapered autumn to spring plant crop fruit [19].The pineapple blossom develops parthenocarpically into a large fruit formed by the complete fusion of 100-200 berry-like fruitlets. The edible part of the fruit consists mainly of the ovaries, the bases of sepals and bracts, and the cortex of the axis[20].

The fruit shell is primarily composed of sepal and bract tissues and the apices of the ovaries Depending on whether the flowers have been pollinated or not, small hard seeds or traces ofundeveloped seed may be present. Fruits are not normally dispersed and in commercial plantations seeds are not produced. Under natural conditions, since the fruit develops parthenocarpically, undeveloped, whitish transparent seeds may be present. Seeds are small, approximately 3-5&1-2mm in size with a rough and tough brown testa, hard and firm endosperm, and a tinyembryo [21].

**Phytoconstituents present in pineapple peel**

Polyphenols are a class of compounds that are found in many plant foods which includes flavonoids, phenolic acids, lignans, stilbenes etc. It is beneficial to health by reducing blood sugar, increasing insulin sensitivity, decreasing cancer risk, reducing inflammation, improving digestion etc. The parts that are known as waste of pineapple are Crown, Peel, stem, and Core. As example citric acid and ferulic acid can be found in pineapple leaves, bromelain can be found in leaves, stem, and peel, ascorbic acid in the core, etc [22-23]. Some polyphenols such as myricetin, salicylic acid, tannic acid, trans-cinnamic acid, and p-coumaric acid are identified in a high dietary fiber powder from pineapple shell, a part of the waste. Components like p-coumaric acid, Syringic acid, p-hydroxybenzoic acid, Vanillin, Catechin, Epicatechin, Caffeic acid, Malic acid and Cinnamic acid are found in pineapple waste. Chromatographic methods play a crucial role in the identification of new and groundbreaking medicinal and biomedical compounds.

**Pharmacological attributes of the plant**

**Anti-inflammatory activity**

*Ananas comosus* and its extracts have been widely used clinically as anti-inflammatory agents in rheumatoid arthritis, soft tissue arthritis, soft tissue injuries, chronic inflammation, chronic pain, and asthma and are currently used in different countries [24]. The major mechanism of action of bromelain is due to its proteolytic nature like immunomodulatory and hormone-like activity via intracellular pathways. In Vitro studies show that bromelain can inhibit pre-incubated with medium alone (PMA)-incubated T cell production of the Th2 cytokine IL-4 and to a lesser degree the Th1 cytokine IL-2 and induced interferon-gamma (IFN)via modulation of the extracellular regulated kinase-2(ERK-2) intercellular signaling pathway (Mynott et al.1999). Bromelain has also been shown to reduce cell surface receptors such as hyaluronan receptor CD44, which is associated with leukocyte migration and induction to pro-inflammatory mediators[25]. Bromelain significantly reduces CD4 T lymphocytes which are primarily effectors in animal models of inflammation. Studying the effect of bromelain having other on postoperative defecation in rats supports findings that the proteolytic activity of bromelain in the colonic micro-environment is not only responsible for its, anti-inflammatory activity but may be involved in the improvement of post-operative ileus. That orally While studying bromelain, researchers at QIMR reported that administered bromelain retains its proteolytic activity, the discovery of two proteins they named CCS and CCZ was previously documented only in the small intestine and found that they could block the growth of a broad range of pigs [26].

**Cardiovascular activity**

Bromelain prevents and minimizes the angina pectoris and transient ischemic attack (TIA). It is useful in the prevention and treatment of thrombophlebitis. It is also used to break down the cholesterol plaques and exerts a potent fibrinolytic activity. Cardiovascular diseases include disorder in blood vessels and heart, coronary heart disease (heart attack), cerebrovascular disease (CVDs or stroke), raised blood pressure(hypertension), peripheral artery disease, congenital heart disease, rheumatic heart disease. Bromelain is effective in the platelet aggregation as a result minimizing the risk of arterial thrombosis and embolism [27]. Bromelain was found to attenuate development of allergic airway disease (AAD) while altering CD4+ to CD8+ T-lymphocyte population. Bromelain was proved to exhibit the ability of inducing cardio protection against ischemia reperfusion injury through Akt/Foxo pathway in rat myocardium [28].

**Antineoplastic activity**

Recent studies have shown that bromelain affects malignancy due to its effect on key pathways that support malignancy. Most of the *in vivo* and *in vitro* studies on the anticancer activity of bromelain are concentrated on mouse and human cells, both cancerous and normal. Beez et al chemically induced mouse skin papilloma was treated with bromelain and they observed that bromelain reduced tumor formation, and tumor volume and caused apoptotic cell death [29-30]. In another study it was found bromelain treated gastric carcinoma cell lines, significant reduction of cell growth was observed, another study proved that bromelain reduced the invasive capacity of glioblastoma cells and reduced *de novo* protein synthesis [30]. Bromelain was found to increase the p53 the well-known activator of apoptosis. It also decreases the activity of cell survival regulators and thus promotesapoptic cell death in tumors.

Bromelain markedly decreases antitumor activity for the following cell lines like leukemia, sarcoma, Ehrlich ascetic tumour, Lewis lung carcinoma and mammary adenocarcinoma [31]. In these studies, i.p (intraperitoneal) administration of bromelain after 24hrs of tumour cell inoculation resulted in tumour regression [32]

**Effect on immunogenicity**

Bromelain has been recommended as an adjuvant therapeutic approach in the treatment of anti-inflammatory, malignant and autoimmune disease [33]. Bromelain also induces surface adhesion and induces the secretion of IL-1β, IL-6 and tumour necrosis factor α (TNF-α) by peripheral blood mononuclear cells. Bromelain can block the Raf-1/Extracellular regulated kinase (ERK-)2 Pathways by inhibiting the T-cell signal transduction. Treatment of cells with bromelain decreases the activation of CD4(+) T cells and reduces the expression of CD25[34]. Bromelain also produces certain effects against rheumatoid arthritis, which is one of the most common autoimmune diseases [35].

**Wound healing activity of Bromelain in Debridement of burns**

If there is a wound in case of burn and formation of wound in second/third degree burn is termed as debridement. Bromelain applied as cream (35% bromelain in a lipid base) can be beneficial for debridement of necrotic tissue and acceleration of healing. Bromelain contains a special enzyme escharase which is responsible for this effect [36]. Escharase is non proteolytic and has no hydrolytic activity against normal protein substrate or various glycosaminoglycan substrates. Its activity varies greatly with different preparation.The study was carried out in procrine model using different bromelain-based agents namely Debriding Gel Dressing (DGD) and Debrase gel showing rapid removal of necrotizing cells and preservation of the unburned tissues [37].

**Effect of bromelain in surgery**

Bromelain has prophylactic action before surgery because it can reduce the average number of days for the complete disappearance of pain and post-surgery inflammation [38]. Different studies have shown that bromelain has shown efficacy in reducing swelling, bruising and pain in wounds having episiotomy. Nowadays, bromelain is used for treating acute inflammation and sports injuries [39].

**Effect of bromelain on Osteoarthritis**

Osteoarthritis is the most common form of arthritis in western countries, In the USA and India prevalence of osteoarthritis ranges from 3.2-33% depending on the joint pain [40]. A combination of bromelain. trypsin and rutin were compared to diclofenac in 103 patients with Osteoarthritis of the knee. After six weeks both treatments resulted in significant and similar reductionsin pain and inflammation. It may play an alternative role against NSAIDS and have analgesic properties which are due to the results of pain mediators like bradykinin. The earliest reported studies investigating bromelain were a series of moderate or severe rheumatoid or osteoarthritis [41-42].

**Hepatoprotective activity**

In an animal model in which alcohol-induced liver injury occurred, the ethanolic and aqueous extracts from *A. comosus* fruits exhibited hepatoprotective action. The ethanolic and aqueous extracts of *A. comosus* contain terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, and polyphenolic compounds, which the authors claim are responsible for this species' hepatoprotective properties. However, additional research is required to determine specific phytochemicals responsible for this organo-protective action [43].

**Antimicrobial activity**

Using the cup-plate method, the antibacterial activity of aqueous and ethanolic extracts of *A. comosus* Extract juice against an isolated UTI pathogen was examined.Fruit extract exhibits substantial antibacterial activity against *Klebsiella, Enterococcus faecalis*, *Bacillus cereus*, and *Staphylococcus aureus*, according to the P value from the One Sample T Test [44].

**Pharmaceutical Formulations on *Ananas comosus***

In a study, pineapples were gathered in Indonesia's West Java province's Subang district. The pineapple crown from the Subang district was found to contain bromelain, which has been shown to have medium antioxidant and immune stimulant action and a protease activity of 7.72±0.45 IU/mg These results demonstrate the potential for developing crude bromelain into herbal remedies. Wet granulation was used to create crude bromelain granules with povidone serving as the binder. The purpose of this study was to create and assess crude bromelain granules from the Subang pineapple crown [45]. Wet granulation was used to create the granule, which is made up of lactose monohydrate, 3% povidone, 1% magnesium stearate, 1% talc, and crude bromelain. The weight of lactose monohydrate was derived by 100% subtraction from the weight of all other components. The mixture was given ethanol to create a wet mass. The damp mixture was filtered through a 1.4 mm sieve before being dried in the drier for 4 hours at 40±0.5 °C followed by its physicochemical characterization [46]. Upon analyses of raw bromelain granules with respect to the moisture content, repose angle, bulk density, tapped density, Hausner's ratio, and compressibility index, it was found that all the results are extremely satisfactory [47]. The granule's moisture content, 4.1 ±0.2%, was within the acceptable range for granules. The risk of cohesion and adhesion will rise as the moisture content enhances [48].A review of the Pineapple plant (Ananas comosus) and its uses from historical ages to the present day.

In another study, sunscreen microemulsion was prepared, from the extracts of pineapple, and its synergistic efficacy on sun protection factor was investigated [49]. Tween 80 and water make up the microemulsion's water phase, while Span 80, PEG 400, and VCO are present in the oil phase. A homogeneous mixture of preheated Tween 80 and water at 50°C was used to prepare the water phase. With the use of a magnetic stirrer, the oil phase was created by combining Span 80, PEG 400, and VCO in a beaker glass. Pineapple peel extract was then uniformly added to the mixture. After adding the water phase solution, the mixture was heated at 50 °C while being stirred magnetically at 1000 rpm for about 30 minutes. To identify the occurrence of substantial alterations in the final preparation, organoleptic observation was carried out. The dosage form that satisfies the requirements has a solution-like consistency, a unique odor, and is translucent or clear [50]. Formula 0 (0% SPF) has an SPF value of 1.8694, which is considered to provide minimal protection. Although formula 1 (5%) had an SPF score of 2.0294, the FDA states that this formula can only offer a very limited level of sun protection. Formula 2 (10%) obtained an SPF value of 2.1391 and, like Formula 3, (15%) obtained an SPF value of 2.9322, both of which indicate that they can offer only moderate skin protection from the sun. Although formula 4 (20%) obtained a value of 3.7759, the value is considered as coming from sun exposure according to the FDA, as a bare minimum of defense. The results of analyzing the SPF values of microemulsion preparations using the Kolmogorov-Smirnov test normality test indicated significant results of 0.216 > 0.05, indicating that the data has a normal distribution of data. The next stage is a homogeneity test using the test Levene, which indicates a significance of 0.180 > 0.05, indicating that the data has a homogeneous data distribution [51-53].

For topical nano cream, this extract has also been used in the formulation of topical nano cream, after due optimization. Phase inversion temperature (PIT) and probe ultrasonication, which combine low and high energies, were used to create triplicate optimum (O/W) nano creams. The oil phase and the aqueous phase were prepared separately, with the oil phase being made up of a mixture of OO (1%), GSO (12%), and T80 (12.63%), and the aqueous phase being made up of distilled water (74.37%) and XG (2%). A beaker holding the aqueous phase was heated to 70°C with the oil phase added drop wise while being stirred at 600 rpm. *Ananu scomosus* peel extract (AcPE) (10%) was then added to the mixture. The coarse emulsion was ultrasonically sonicated for five minutes at a frequency of 20 kHz while simultaneously being added to Phy-Et (1%) and scented oil (2%). After subjecting it to the characterization tests like pH, Particle Size, PDI for Accelerated Stability, Coalescence Rate, Ostwald Ripening Rate, Microbiological limit test, and heavy metal test, it was concluded that the Ostwald ripening instability phenomena at 4°C at 6 weeks of storage had a considerable impact on the ideal nanocream, although coalescence had little to no effect. The results confirmed that the AcPEnano cream was secure and uncontaminated by microorganisms or heavy metals. -Thus, these characteristics demonstrated the nanoemulsion's appropriateness for topically applying to skin. In summary, the study's improved AcPEnano cream, which concentrated on naturally derived plant active components, may find use in the cosmeceutical sector [50-51].

## CONCLUSION

It has been found that *Ananascomosus*hasa plethora of pharmacological activities, that has been used from the historical ages to the present day. Owing to the presence of multiple bioactive constituents in various parts of the plant renders its significance in the field of research and development. Its extract has already been investigated in the treatment of disorders, where positive outcomes have paved the way towards its utilization in pharmaceutical formulations. Variousformulationshave been implemented using the plant extract, as well as with the isolated bioactive component, where the presence of active phytoconstituentshas shown remarkable positive effects. The phytochemicals present in *Ananas comosus* are usedfor the treatment of wound healing, ulcers, cardiovascular diseases, osteoarthritis, inflammation, and even contributeto the remission of surgical wounds.Thus, it can be rightly concluded that the implementation of this magic fruit in the pharmaceutical field has potentially replaced synthetic drugs and formulations and reduced the side effects of conventional synthetic drugs, propounding that traditional and herbal drugs if explored for application in drug delivery system, can cater to multiple needs of the therapeutic aspects.

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**REVIEW ARTICLE**

# A Review of the Bounds on the Spectral Radius and the Principal Eigenvector of a Graph in Terms of Various Parameters

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**ABSTRACT**

In spectral graph theory, there has been significant emphasis on estimating the spectral radii of graphs. Similarly, estimating the components and their ratios of the principal eigenvector of a graph has piqued the interest from mathematicians due to its relevance in spectral graph theory. Numerous well-documented research articles address these topics. This survey reviews the bounds on the spectral radii and on the components and ratios of the components of the principal eigenvectors of graphs. The article also briefly explores the various parameters involved in establishing these bounds.

**Keywords:** Adjacency matrix, Spectral radius, Principal eigenvector

## INTRODUCTION

Graph theory, a branch of discrete mathematics, studies mathematical structures known as graphs, with its origins stemming from the famous Königsberg Bridge Problem [16]. Spectral graph theory, a subfield that emerged in the late 1950s and early 1960s, has seen substantial development since then. Significant research publications have contributed to its growth, exploring graph properties by associating them with various matrices such as adjacency matrices, incidence matrices, Laplacian matrices, signless Laplacian matrices, Distance Laplacian matrices, and maximum degree matrices, employing techniques from linear algebra. In spectral graph theory, mathematicians primarily investigate characteristic polynomials, eigenvalues, and eigenvectors of these matrices to uncover structural properties of graphs. Eigenvalues of graph-associated matrices find extensive use in both intra and interdisciplinary applications. For instance, the adjacency matrix concisely represents a graph's structure, facilitating analysis and exploration of its properties. This matrix underpins many graph algorithms like Depth-First Search

(DFS), Breadth-First Search (BFS), and Dijkstra's shortest path algorithm. It finds application in network analysis, such as in communication and transportation networks. Additionally, in electrical engineering, the adjacency matrix aids in circuit analysis, assisting in the study of electrical properties and the calculation of currents and voltages. The Laplacian matrix is instrumental in sensor networks and machine learning algorithms. For instance, in sensor networks, the ratio of the second smallest eigenvalue to the largest eigenvalue of the Laplacian matrix determines the convergence speed of decision fusion algorithms, thereby enhancing network performance. Machine learning algorithms also leverage the Laplacian matrix for tasks such as clustering and dimensionality reduction.Moreover, the applications of graph-spectra extend beyond these fields to diverse disciplines such as physics, medical science, quantum chemistry, cryptography, biology, and economics. This broad applicability underscores the importance of spectral graph theory in understanding and solving real-world problems across various domains. On the other hand, eigenvectors especially the principal eigenvectors of graphs have practical applications in various fields, for example the eigenvector associated with the second-largest eigenvalue of a connected graph has been utilized in spectral partitioning algorithms [34]. Initially, the PageRank algorithm used by Google was based on the principal eigenvectors of graphs [2]. Some other applications of this vector can be found in [38] and references there in.

The adjacency matrix is considered one of the early introduced matrices associated with graphs. The study of the spectra of adjacency matrices encompasses a wide range of topics, including the study of the number of distinct eigenvalues of graphs, the estimation of graph parameters in terms of eigenvalues, co-spectrality, estimating the eigenvalues and the energy of graphs, the research of limit points of the eigenvalues of graphs, and ordering of graphs using eigenvalues, estimation of extreme components of the principal eigenvectors, etc. The adjacency matrix's spectrum provides valuable insights into the characteristics of a graph. For example, graph $G$ is regular if and only if the maximum degree, spectral radius, and minimum degree of the graph are equal, again if the spectral radius is the only positive eigenvalue of a graph $G$, then $G$ is complete multipartite, and vice-versa, etc. Despite the importance of a graph's spectral radius and other eigenvalues, their precise values are not always known. Therefore, it is crucial to establish the bounds (upper and lower) on a graph's eigenvalues. Since 1957, the process of establishing bounds on the eigenvalues, especially spectral radius, has been underway. These bounds often rely on graph parameters such as order, size, degrees of the vertices, chromatic number, clique number, independence number, girth, number of certain walk lengths, etc. On the other hand, since 2000 there has been a growing emphasis on estimating the components and ratios of the components of the principal eigenvector of a graph, as they reveal various graph properties. For example, if the maximal component of the principal eigenvector is$\frac{1}{\sqrt{2}}$, the graph is identified as a star graph.Likewise, a graph is a regular graph if and only if the ratios of the components of the principal eigenvector are equal to 1, etc. In fact, the ratios of the components of the principal eigenvector of a graph are considered to be the measure of the regularity of the graph. The estimations on the components and ratios of the components of the principal eigenvector of a graph are constrained by graph parameters, such as the order, size, maximum, minimum, and average degrees, spectral radius, etc.It is to be noted that these kinds of bounds not only aid in estimating the precise values of the eigenvalues but also provide insight into their interrelationships.

Until and unless specified by a graph $G$ we mean a simple connected undirected and finite graph with vertex set $U(G)$ and edge set $E(G)$, respectively. By order and size of a graph $G$ we mean cardinalities of$|U(G)|$ and$|E(G)|$, respectively. It is to be noted, unless otherwise specified, $G$'s order and size are denoted by $n$ and $e$, respectively. For $i = 1,2,\dots,n$, the degree $d(u_i)$ of a vertex$u_i \in U(G)$ is the number of vertices$u_j\,(i \neq j)$adjacent to$u_i$, i.e.$u_j u_i \in E(G)$. Again, $\Delta$ (= $\max\{d(u_i)|u_i \in U(G)\}$), $\delta$ (= $\min\{d(u_i)|u_i \in U(G)\}$) and$\bar{d} = \frac{2e}{n}$ are the maximum, minimum and the average degrees of a graph $G$, respectively. The adjacency matrix $A(G) = [a_{ij}]_{n\times n}$ is defined as$a_{ij} = 1$, if$u_i u_j \in E(G)$ and$a_{ij} = 0$ if$u_i u_j \notin E(G)$. The eigenvalues of $A(G)$ are also known as the eigenvalues of $G$, and they are denoted here by$\tau_i\,(i = 1,2,\dots,n)$, where$\tau_1 > \tau_2 \geq \tau_3 \geq \cdots \geq \tau_n$. Energy$E_G$ of a graph $G$ is defined as$\sum_{i=1}^{n}|\tau_i|$. The orthonormal eigenvectors corresponding to the eigenvalues$\tau_i\,(i = 1,2,\dots,n)$ are denoted here by$Y_i = (y_{i_1}, y_{i_2}, \dots, y_{i_n})^T\,(i = 1,2,\dots,n)$. According to the Perron-Frobenius theorem (Pillai, Suel, and Cha, 2005), there exists a unique eigenvalue $\tau_1$ of $A(G)$ with algebraic multiplicity 1,which is positive andis known as the spectral radiusof $G$. Additionally, there is a unique eigenvector associated with $\tau_1$ that has unit length and all positive real components. This unique eigenvector is called

the principal eigenvector of $G$ and is represented by$Y_1 = \left(y_{1_1}, y_{1_2}, .., y_{1_n}\right)^T$. The maximal and the minimal components of the principal eigenvector$Y_1$ are denoted here by$y_{max}$ and$y_{\min}$, respectively. For basic definitions and results we refer to [39].

**Survey on the bounds of the spectral radius of a graph**

Doob [14] raised some of the fundamental problems of a graph related to the eigenvalues of the adjacency matrix. The monograph "Spectra of graph" [15] is one of the important books, as it provides an overview of nearly all studies pertaining to the theory of graph spectra published before 1979. Later, this book was updated by Cvetković et al. [10], [12].

Researching the estimation of eigenvalues, especially the spectral radius of a graph, holds considerable importance in spectral graph theory. Collatz and Sinogowitz [36] made significant contributions to this field by establishing bounds on the spectral radius$\tau_1$ of a graph $G$, a work widely acknowledged as seminal in eigenvalue estimation. Their paper introduced both an upper bound$\tau_1 \leq n-1$ and a lower bound $2\cos\frac{\pi}{n+1} \leq \tau_1$ on the spectral radius$\tau_1$, relying on the graph's order $n$. Furthermore, they derived an additional bound$\tau_1 \leq \sqrt{n-1}$ for a tree that of order $n$. Several early research works concerning the spectral radius$\tau_1$ with respect to vertex-degrees$d(u_i)$, maximum degree$\Delta$, average degree$\bar{d}$, and minimum degree $\delta$, are documented in Cvetković and Rowlinson [11] such as$\delta \leq \bar{d} \leq \tau_1 \leq \Delta$, $\sqrt{\frac{\sum_{i=1}^{n} d(u_i)^2}{n}} \leq \tau_1$, and$\sqrt{\Delta} \leq \tau_1$, etc. It's important to note that the bound $\delta \leq \bar{d} \leq \tau_1 \leq \Delta$ emerged from reformulated inequalities originating from non-negative matrix theory [28]. Nosal [31] set the bounds $n-1 \leq \tau_1 + \bar{\tau_1} \leq \sqrt{2}n - \sqrt{2}$for the spectral radii of a graph $G$ and that of its complement$\bar{G}$ in terms of their order $n$.

Brigham and Dutton [4] introduced bounds on the spectrum of a graph, considering factors like the numbers of positive and negative eigenvalues, independence number, order, size, etc. Furthermore, they refined these bounds for some specific classes of graphs such as triangle-free graphs, bipartite graphs, etc. Favaron et al. [17] focused on improving some classical bounds concerning the spectral radius of a graph. In this article, they proved some conjectures regarding the eigenvalues of a graph with order $n$, particularly concerning the spectral radius, such as minimum (dual degree)$\leq \tau_1 \leq$ maximum (dual degree), etc. They also established inequalities$|\tau_1| + |\tau_i| \leq 2\sqrt{e}$,$|\tau_i| \leq \frac{e}{\tau_1}$, and $n - \chi^* \leq \sum_{i=1}^{\chi^*} \tau_i$, etc. where $i = 1,2,\ldots,n$, and$\chi^*$ is so called "mischromatic number". Brualdi and Hoffman [6], Stanley [35], Friedlandand Yuan [19]contributed along the same line utilizing the graph parameters order and size of a graph.

Li [27] and Zhou [44]established bounds on the sum of the spectral radii of a graph and its complement. Li [27] introduced the Nordhaus–Gaddum type upper bound$\tau_1 + \bar{\tau_1} \leq \sqrt{1 + 2n(n-1) - 4\delta(n-1-\Delta)} - 1$ on the spectral radii$\tau_1$ and$\bar{\tau_1}$ of a graph $G$ and its complement$\bar{G}$, respectively based on order $n$, maximum degree $\Delta$ and minimum degree $\delta$ of $G$.Li [27] and Zhou [44], independently established the inequality$\tau_1 + \bar{\tau_1} \leq \sqrt{2(n-1)(n-2)}$ in terms of the order $n$ of a graph and that of its complement. Similarly, Hong and Shu [23, 24]developed Nordhaus–Gaddum type bounds for the spectral radii$\tau_1$ and$\bar{\tau_1}$ of graphs $G$ and$\bar{G}$, respectively. Hong and Shu [23] concentrated on improving previously defined Nordhaus–Gaddum type bounds for the spectral radii of graphs. Moreover, the same article comprised two inequalities$\tau_1 + \bar{\tau_1} \leq \sqrt{\left(2 - \frac{1}{k}\right)n(n-1)}$ and$\tau_1 + \bar{\tau_1} \leq \left(2 - \frac{1}{K}\right)n(n-1)$, in terms of the chromatic numbers $\chi$ and$\bar{\chi}$ of $G$ and its complement $\bar{G}$, respectively, where $k$ =min$\{\chi, \bar{\chi}\}$ and $K$ =max$\{\chi, \bar{\chi}\}$. Similarly, on the basis of the same parameters $\chi$ and$\bar{\chi}$ Hong and Shu introduced$\tau_1 + \bar{\tau_1} \leq \sqrt{2 - \frac{1}{\chi} - \frac{1}{\chi}}$ in Hong and Shu's article [24].

Cao [7] presented the bound$\tau_1 \leq \sqrt{2e - \delta(n-1) + \Delta(\delta - 1)}$ for a graph $G$ of order $n$, size $e$ and maximum and minimum degrees $\Delta$ and $\delta$, respectively. Moreover, using the maximum 2-degree$T^*$ of the vertices of $G$, Cao also proved$\tau_1 \leq \sqrt{T^*}$. Again, Hu [25] obtained the bound$\tau_1 \leq \sqrt{2e - n - \delta + 2}$. Hong et al. [22] established a bound$\tau_1 \leq$

$\frac{\delta-1+\sqrt{(\delta+1)^2+4(2e-\delta n)}}{2}$ on the spectral radius $\tau_1$ of a graph in terms of its order $n$, size $e$, and the minimum degree $\delta$. Berman and Zhang [2] also estimated the spectral radius of a graph using its degrees of vertices and the number of cut vertices. Yu et al. [40] gave two lower bounds for the spectral radius of the adjacency matrix and that of the Laplacian matrix in terms of the degrees of vertices of a graph. Considering $l(u_i)$ to be the average degrees of the vertices adjacent to $u_i$ in a graph $G$, Das and Kumar [13] presented the inequality $\tau_1 \leq \max\left\{\sqrt{l(u_i)l(u_j)}\middle| u_iu_j \in E(G)\right\}$ for $\tau_1$. In addition, they also established $\tau_1 \geq \sqrt{\frac{(\Delta+d_j{}^*-1)+\sqrt{(\Delta+d_j{}^*-1)^2+4(\Delta-1)(d_j{}^*-1)+4c_{1j}{}^{*2}+8c_{ij}{}^*\sqrt{\Delta}}}{2}}$, where $u_1$ is the vertex of degree $\Delta$, $d_j{}^*=\max\{d(u_k)|u_lu_k \in E(G)\}$ and $c_{ij}{}^*$ is the cardinality of the common neighbor between the vertices $u_1$ and $u_j$. The same article included some other bounds on the second-largest eigenvalue $\tau_2$ and the smallest eigenvalue $\tau_n$ of $G$. Again Nikiforov [29] proved bounds $\tau_1{}^r \geq \frac{W_{q+r}}{W_q}$ and $\tau_1{}^r \leq \frac{\omega-1}{\omega}W_r$, etc. for the spectral radius $\tau_1$ and its powers using the concept of total number of certain-length of walks such as $W_{q+r}, W_q$, and $W_r$ and clique number $\omega$ of a graph. He also generalized a number of other bounds of the spectral radius and characterized so-called pseudo-regular graph and pseudo-semi-regular graph in spectral terms. Later, Bollobás and Nikiforov [3] proved some relations between the spectral radius and the number of cliques contained in a graph. Feng et al. [18], mainly using the average 2-degree $m_{u_i}$ of a vertex $u_i$, maximum degree $\Delta$, and the maximum 2-degree $T^*$, obtained some estimations for $\tau_1$ such as $\tau_1 \leq max_{u_i \in U(G)}\sqrt{\frac{d(u_i)^2+d(u_i)m_{u_i}}{2}}$, $\tau_1 \leq max_{u_iu_j \in U(G)}\sqrt{\frac{d(u_i)\{d(u_i)+m_{u_i}\}+d(u_j)\{d(u_j)+m_{u_j}\}}{2}}$, $\tau_1 \leq \frac{\Delta+\sqrt{8T^*+\Delta^2}}{4}$, $\tau_1 \leq max_{u_i \in U(G)}\frac{d(u_i)+\sqrt{d(u_i)m_{u_i}}}{2}$, etc. for a graph $G$. Cioabă et al. [9] determined a lower bound $\tau_1 \leq (n-1)^{1/D}$ for the spectral radius $\tau_1$ of a graph in terms of the number of $n$ vertices and the diameter $D$ of the graph. The article "Bounds of the Spectral Radius and the Nordhaus-Gaddum Type of the Graphs" by Wang et al. [37] can be considered a significant research article regarding estimating the spectral radius of the adjacency $A(G)$ and that of Laplacian matrix $L(G)$ in terms of various parameters. Wang et al. [37] established several estimations for $\tau_1$ in terms of parameters such as a graph $G$'s second maximum degree $\Delta^*$, the second minimum degree $\delta^*$, the average 2-degree $m_{u_i}$ of a vertex $u_i$ contained in $G$, number of $K_3$ associated with a vertex $u_i$ (denoted by $T_{u_i}$), number of the vertices adjacent to $u_i$ ( i.e. $|N_{u_i}|$), number of vertices $z_1$ and $z_2$ with degrees $\Delta$ and $\delta$, respectively, etc. other than the maximum degree $\Delta$, minimum degree $\delta$, number of vertices $n$, and edges $e$ contained in $G$. Some of the bounds on $\tau_1$ comprised in "Bounds of the spectral radius and the Nordhaus-Gaddum type of the graphs" are $\tau_1 \leq max_{u_iu_j \in E(G)}\frac{d(u_i)^2m_{u_i}+d(u_j)^2m_{u_j}-2T_{u_i}-2T_{u_j}}{2\left(d(u_i)+d(u_j)-\left|N_{u_i}\cap N_{u_j}\right|\right)}$, $\tau_1 \leq \sqrt{\Delta(\delta^*-1)+2e-z_2\delta-(n-1-z_2)\delta^*}$, and $\tau_1 \geq \sqrt{\delta(\Delta^*-1)+2e-z_1\Delta-(n-1-z_1)\Delta^*}$, where $\Delta \leq min\{n-1-z_1, n-1-z_2\}$. Again the same article provided another bound $\tau_1 \leq \frac{\delta^*-1+\sqrt{(\delta^*-1)^2+8e-4z_2(\delta-\delta^*)-4n\delta^*}}{2}$ with the condition $\Delta \leq n-1-z_2$. Additionally, T. Wang et al. introduced a Nordhaus-Gaddum-type upper bound for the Laplacian matrix also.

It is to be noted that mathematicians also emphasize studying various graph-related operations and their impacts on the spectra of graphs. The article "Spectra of graphs resulting from various graph operations and products: a survey" by Barik et al. [1] summarized some of these studies conducted on graph operations and the spectra of graphs. In essence, Barik et al. [1] provided a summary of known results regarding the spectra of graphs emerging from various graph operations. It included a detailed discussion of the structure of the associated eigenvectors together with an explicit description of the eigenvalues of the resulting graphs.

**Survey on the bounds of the components and the ratios of the components of the principal eigenvector of a Graph**

Since the year 2000, research in spectral graph theory has expanded beyond just estimating graph spectra to include an interest in the eigenvectors of graphs, particularly the principal eigenvectors, due to the insights they provide into various graph characteristics. Estimating the components and their ratios of the principal eigenvector has become a major focus for mathematicians. Britta Papendieck and Peter Recht [32] were early pioneers in this area, exploring

whether the principal eigenvector can reveal structural information about a graph. They estimated an upper bound on the maximum component of the principal eigenvector using the $l$ -norm($l \in [1,\infty]$ as$\left(\frac{\tau_1{}^{l-2}}{1+\tau_1{}^{l-2}}\right)^{1/l}$ for the maximal component of that vector. Similarly, Zhao and Hong [43] also focused on the $l$ -norm($l \in [2,\infty]$ of the principal eigenvector of a graph, and obtained upper bound$\left(\frac{(n-1)^{\frac{l-2}{2}}}{1+(n-1)^{\frac{l-2}{2}}}\right)^{1/l}$ and lower bound $\left(\frac{1}{n}\right)^{1/l}$ on the maximal component of that vector in terms of the graph's order $n$.Later, following their works, Zhang [42], Cioaba and Gregory [8], Nikiforov [30], Goldberg [20], etc. also developed several bounds on the components as well as the ratios of the components of the principal eigenvector of a graph. Zhang [42] established bounds on the ratio$\frac{y_{max}}{y_{min}}$ for a non-regular graph such as$\frac{(\Delta-\bar{d})(\tau_1-\delta)}{(\Delta-\tau_1)(\bar{d}-\delta)} \geq \frac{y_{max}}{y_{min}}$ and$\frac{y_{max}}{y_{min}} \geq \sqrt{\frac{\Delta}{\delta}}$ in terms of the graph's maximum degree $\Delta$, minimum degree $\delta$, average degree$\bar{d}$ and spectral radius$\tau_1$. In addition, using the graph parameters such as the order $n$, maximum degree $\Delta$, minimum degree $\delta$ and diameter $D$, a sharp upper bound$\tau_1 < \Delta - \frac{\Delta+\delta-2\sqrt{\delta\Delta}}{nD\,\Delta}$ on the spectral radius$\tau_1$ of a non-regular graph is also introduced in the same article. Cioaba and Gregory also focused on estimating and improving bounds on the extreme components$y_{max}$ , and$y_{min}$ of the principal eigenvector of a graph and the ratio$\frac{y_{max}}{y_{min}}$ in [8]. While defining those bounds they mainly utilized parameters such as the number of $n$ vertices and $e$ edges, degrees of vertices $d(u_i)$, maximum $\Delta$ and minimum $\delta$ degrees, spectral radius$\tau_1$, etc. In [30], Nikiforov introduced a bound on the ratio$\frac{y_{1_j}}{y_{1_i}}$ of the components$y_{1_j}$ and$y_{1_i}$ of the principal eigenvector$Y_1$ of a graph in terms of the distance between the two vertices associated with the corresponding components$y_{1_j}$ and$y_{1_i}$, and the spectral radius$\tau_1$ of the graph. On the other hand, Goldberg [20] obtained a lower bound for all of the components of the principal eigenvector of a non-regular graph in terms of degrees of vertices and spectral radius.

## CONCLUSION

The above discussion highlights that mathematicians have developed different spectral bounds for the adjacency matrix and have been keen on deriving new bounds regarding eigenvalues, particularly for the spectral radius of a graph based on various parameters. Later, mathematicians have delved into estimating eigenvalues, especially the spectral radii of matrices such as the maximum degree matrix, minimum degree matrix, sparse random matrix, Wigner matrix, Randic matrix, etc., compared to the adjacency matrix. However, there remains a conviction that there is room for advancing this domain by refining previously established bounds and producing new bounds on the spectral radius concerning various graph parameters. It is also noted that certain previously introduced bounds on the spectral radii demonstrate superior performance for specific classes of graphs. Hence, there is an opportunity to formulate new bounds that offer nearly precise values not for a particular class of graphs, but for all graphs. We believe it is possible to establish new bounds on the components and their ratios in the principal eigenvectors of graphs by introducing parameters beyond those previously mentioned. Additionally, there are currently few lower bounds for the minimal component of a graph's principal eigenvector, indicating that there is room for developing such bounds. Discovering new practical applications for the principal eigenvectors of graphs could significantly advance spectral graph theory. Therefore, research into establishing bounds for the spectral radius and the components and ratios of the components of a graph's principal eigenvector is ongoing, with expectations for continued exploration in the future.

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**RESEARCH ARTICLE**

# Blind People's Bus Route Identification System

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**ABSTRACT**

This study offers by RFID-based bus detection system designed for traveling and for making a movement to any place is not challenging for sight folded persons. To make the design of proposed system of a system database ,website make up and it has to consist of two detection subsystems on the buses and the station of bus consists of other. Every adjacent stations are clearly identified by the bus sensing subsystem and thereafter declared by an audio message sent internally. In addition, the bus subsystem will identify any blind individuals in the vicinity of the station and notify the bus driver of their count. To notify the blind, the bus station subsystem detects approaching buses and announces them inside the station. The entire system prototype has been recommended by testing. The functionality which has been built are safety, cost-effectiveness and are promising for the people who are blind folded

**Keywords:** Automatic Teller Machines, RFID technology, Arduino, embedded systems.

## INTRODUCTION

Our lives are becoming more and more reliant on "embedded systems," or digital information technology specifically pervasive across the surroundings nearer.. Today, embedded systems contain more than 98% of all processors used, making them invisible to the consumer as "computers" onethicalfeeling [1]. An embedded system is a special-

purpose system where the computer is entirely contained within or devoted for the system and the device that it manages. An embedded system, contrary to a regular used computer like a personal computer, carries out one or more pre-defined activities, typically with extremely precise specifications. Because the system is only used for specified functions, design engineers are able to maximize to make product smaller similarly extravagant less [2]. Because of economies of scale, embedded systems are frequently produced in large quantities.Amid the significant advancements in high-end embedded systems in all these years is the growing utilization of PC hardware. This trend has resulted in a sharp decline in the cost of high-end system hardware, opening the door to projects that would not have been possible due to the premium of embedded non-PC technology[3]. However, the embedded PC platform's software options are not nearly as appealing as the hardware. An embedded system usually consists of a single microprocessor board on which the programs are ROM-stored. Embedded systems are used by almost every appliance with a digital interface, including watches, microwaves, VCRs, and automobiles. While many embedded systems are sufficiently specialized that all of the logic can be implemented as a single program, some embedded systems do have an operating system [4-5].In terms of physical form, embedded systems can be found in tiny, portable appliances like MP3 players, digital watches to enormous, traffic signals fitting, controllers in the factories, and controlling equipment's in the nuclear power plant.On these processors, the software used for applications is frequently called firmware. The most basic devices are made up of a single microprocessor, also referred to as a "chip," which can be bundled with additional chips to form an Application Specific Integrated Circuit (ASIC) or hybrid system[6-9]. It receives input from a detector or sensor and outputs to a switch or activator, which can be used to control a valve to regulate the flow of fuel to an engine or to start or stop a machine. Given that an embedded system consists of both hardware and software.

## MATERIALS AND METHODS

An Embedded is a software application it is implemented for a special purpose and it allows the constructed hardware to communicate with the software directly. Features and flexibility are provided by software. The system Hardware tools used are for testing the performance and security assurance are sensors, memory, processors, ASICs. The block diagram of an embedded system as shown in the figure 1. Custom hardware is the foundation of every embedded system, and it is centered byan Central Processing Unit (CPU). In support of it, the software tool has been installed in memory chips are part of this hardware. The term "firmware" also refers to the software that is stored on the memory chip. Details of hardware of embedded system:

- CPU (Central Processing Unit)
- ROM and RAM Memory
- Input and Output Devices
- Communication interface
- Circuitry Specific Applications

**Hardware Requirements**

In order to ensure usability, accessibility, and reliability, multiple hardware components are involved in the design of a bus route identification system for blind individuals. The system block diagram as shown in the figure2.The following crucial pieces of hardware are needed for such a system.

1. Microcontroller
2. EM 18 READER
3. RFID CARDS
4. Driver circuit
5. Voice Record Play back module
6. Speakers

**User interface system**

For a bus route identification system designed for the blind to be both accessible as well as helpful, its interface's components are crucial. These are the key user interface components which typically appear in such a system: text to speech, audio output device, navigation system and feedback.

The voice record process and voice playback module has following steps to code in the ardunio system.

**Voice record process**

1.5V or 12V is given a power supply. Switched the power either to 5Vor 12V.

2. 8 channels have been used (M1 TO M8) for audio recording. 1.3 minutes is a length of recording.

3. For recording on board MIC is automatically used.

4. LED (LD1) on the board is switched on.

5. REC/PLAY is a slide button is used to select the recording or play mode.

6. The channel (M1-M8) is established to record the message in record mode. M1 is connected to Ground to record a message M0 Channel or Hold and Press button M1 .

7. When LED (LD2) is on record mode on the chip records whatever we speak and it will be captured by MIC and recorded. LED (LD2) will be automatically turned off when recording segment is filled.

8. M1 button is pressed to stop recording or it is disconnected from GND.

**Voice playback from module:**

1. For the board Speaker section the speaker is connected.

2. Check recorded. Slide the REC/PLAY to PLAY part.

3. Status LED (LD2) will be ON when (M1-M8) button has pressed. Recording of sound play in the speaker is completed.

4. The Same process repeated for the other entire channel.

## RESULT AND DISCUSSION

In most cases, USB can be used to link a Raspberry Pi to a PC. We may use VNC Viewer to establish a wireless connection between our Raspberry Pi and PC. The Raspberry Pi's IP address must be given to VNC Viewer. With VNC Viewer, we can operate on the Raspberry Pi in any way. The details of a specific tag will be transformed from text to speech through the Speakers package and played back over the speaker whenever the RFID scanner picks up the tag that is attached to the bus. The compiler result will then be displayed in the figure3 below.

They should be granted assistance so that they can live like everyone else. In this study, we demonstrated bus detection system of an RFID for the blind. Not just blind passengers, but all passengers can benefit from the easy-to-use and convenient service offered by the suggested system. The structure consists of bus subsystem and station subsystem .Each and every travelling person are informed of the next stations along bus route by the subsystem of bus. Additionally, the number of sight less passengers that needed the travelling bus and their reached stops will be given to the driver of the Bus. The station subsystem will give information regarding the buss as they reach or near to stop. The planned system's prototype was constructed and tested without incident. In terms of usefulness and performance, our design looks good.

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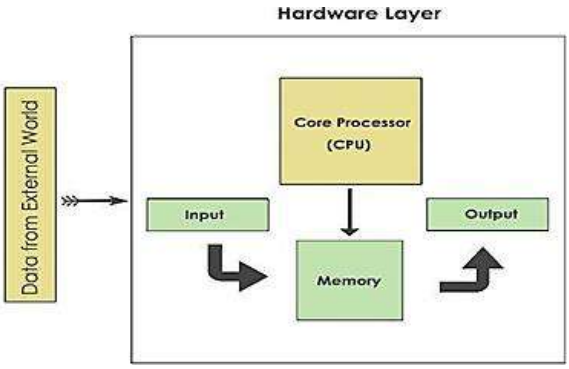


**Figure 1:Block diagram of embedded systems**

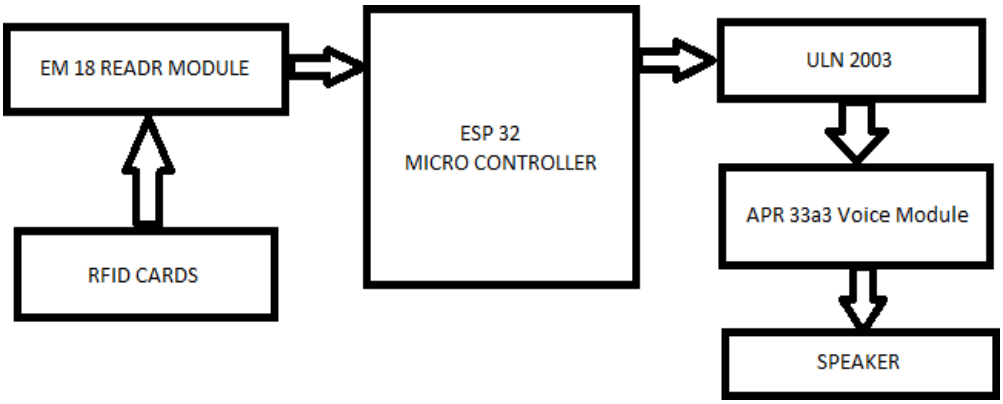


**Figure 2:schematic diagram of hardware**

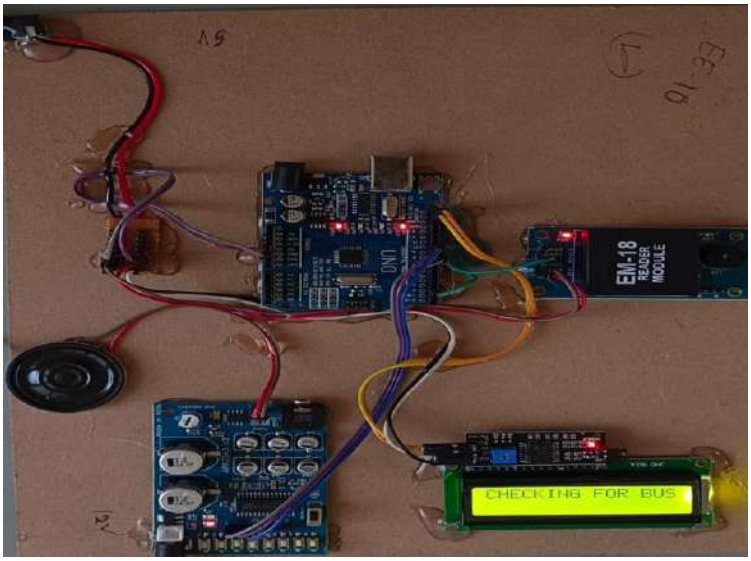


**Figure 3: Hardware prototype kit**

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**RESEARCH ARTICLE**

# Correlation Study between Particle Size and Raman Spectra for Nano Crystalline $Mg_x Mn_{1-x} Fe_2O_4$ prepared by Co Precipitation Technique

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**ABSTRACT**

Nanoparticles having particle size 25 – 40 nm for composition $x$ = 0.0 – 0.5 prepared by coprecipitation method. The microstructure and Raman scattering have been studied by means of Energy dispersive analysis of X-rays (EDAX), X-ray diffraction (XRD), Transmission electron microscopy (TEM), Raman spectroscopy measurements, effect of non – magnetic $Mg^{2+}$ substitution on particle size and Raman scattering for $MnFe_2O_4$ have been explained.

**Key words:** Ferrites, Coprecipitation technique, Transmission Electron Microscopy, X-ray diffraction, Raman spectroscopy.

## INTRODUCTION

The research in the field of ferrites is well cultivated but still researchers found exciting results for ferrites due to particle size reduction, cation redistribution and improved properties [1]. This has renewed interest to study different properties of pure and mixed spinel ferrite systems in nanocrystalline regime Research reports describing effect of grain size on magnetic properties of spinel ferrites synthesized by various chemical routes including ball milling are available [2, 3]. Present work aims to study relationship between particle size and Raman scattering for $Mg_xMn_{1-}$

$_xFe_2O_4$ ($x$ = 0.0 – 0.5) synthesized by co-precipitation technique. Manganese ferrite ($MnFe_2O_4$) and magnesium ferrite ($MgFe_2O_4$) are partially inverse spinels and their degree of inversion is sensitive to the thermal history of the sample, microstructure and structural parameters [1].

## MATERIALS AND METHODS

The spinel ferrite system $Mg_xMn_{1-x}Fe_2O_4$ with variable compositions $x$ = 0.0, 0.1,0.2, 0.4 and 0.5 was prepared by air oxidation of an aqueous suspension containing $Mg^{2+}$, $Mn^{2+}$ and $Fe^{2+}$ cations in stoichiometric proportions. The starting solutions were prepared by mixing 50ml of aqueous solutions of $MgSO_4.7H_2O$, $MnSO_4.H_2O$ and $FeSO_4.7H_2O$ in proper proportions. A 2M solution of NaOH was prepared as a precipitant. The starting solution (pH ~ 3.5) was added into the precipitant because the solubility product constants for the hydroxides of the cation are exceeded [4, 5] and sequential precipitation of the hydroxides can be avoided. The suspension (pH = 10.5) containing dark green intermediate precipitates was formed. Then the suspension was heated and kept at 60$^o$C temperature, while hydrogen peroxide ($H_2O_2$) was added to promote oxidation reaction until all the intermediate precipitates changed into the dark brownish precipitates of the spinel ferrite (as prepared samples or precursor). The samples were filtered, washed by acetone and dried at 200$^o$C under vacuum (final product). The preparation of ferrite powders by the oxidation method consists of oxidation by air($O_2$) bubbling through an aqueous solution containing ferrous ions ($Fe^{2+}$) and other divalent ions ($M^{2+}$) after an alkaline solution (ROH) has been added, the reaction is given by: $Fe^{2+} + M^{2+} + ROH + O_2 \rightarrow M^{2+}_{1-x} Fe^{3+}_{2-x}O_4$ where ROH is NaOH, KOH, $NH_4OH$ etc. Thus, ferrite powders of high homogeneity and purity are obtained [6, 7]. The stoichiometry of the powdered samples was checked by energy dispersive analysis of X-rays (EDAX). X-ray diffraction data were collected on a Philips PW 1710 automated X-ray powder diffractometer using $CuK_\alpha$ radiation, graphite monochromator, and Xe-filled proportional counter. Micro structural characterization was performed by transmission electron microscopy (TEM), using a TECNAIK20 (Philips) microscope operated at 200 kV. For the TEM observations, the powder was first dispersed in amyl acetate by ultrasonication and then the suspension was dropped on a copper grid with a carbon film. Raman scattering recorded using Horiba LabRAM HR Evolution Raman Spectrometer at IIT Madras.

## RESULTS AND DISCUSSION

The EDAX spectra shown in Figure 1 clearly demonstrate a group of three well defined peaks located between energy 5.5 keV to 7.0 keV. The maximum observed at ~ 6.4 keV is directly related to $FeK_\alpha$, peak at ~ 7.0 keV corresponding to $FeK_{\beta1}$ and peak at~6.0 keV belongs to $MnK_\alpha$ characteristic line. It is important to note that $MnK_\beta$ line interferes with $FeK_\alpha$ line in the spectra [8]. The peak located at ~ 1.254 keV confirms the present of $MgK_\alpha$ characteristic line. Here, care should be taken as $MgK_\beta$ (1.3 keV) line overlaps with $MgK_\alpha$ line. The maximum on the left part of the spectra at ~ 0.5 keV clearly comes from $OK_\alpha$, while very small peak that located at ~ 0.75 keV comes from $FeL_{\alpha1}$ characteristic line. The peak at extreme left of the spectra centered at ~ 0.25 keV connected with carbon ($CK_\alpha$) characteristic line of impurity. The origin of sodium impurity is well understood from the chemical used for the synthesis of spinel ferrite system in which NaOH is used as a precipitant. On the other hand impurity like carbon was likely to have been introduced from carbon coated shield to the pole pieces and special EDAX specimen holder made of carbon, beryllium and aluminum used in EDAX spectrometer [8]. It is well established fact that if a $K_\alpha$ line is identified in a spectrum then a $K_\beta$ line should exist. Furthermore, $K_\beta$ line has intensity 1/10 of intensity of $K_\alpha$ line,i.e. $K_\alpha$:$K_\beta$ = 10 : 1 [9]. In the present case, we have also observed this relationship for $FeK_\alpha$ and $FeK_{\beta1}$ lines in the spectra.

Shown in Figure 2 are the room temperature (300 K) X-ray powder diffraction patterns for the $x$ = 0.0, 0.2 and 0.5 compositions of $Mg_xMn_{1-x}Fe_2O_4$ spinel ferrite system. All the diffraction patterns were indexed and lattice constant value for each composition was determined using 'Powder-X' software. It is seen that the diffraction patterns could be indexed for face centered cubic (*fcc*) structure of space group $O_7^h$ (Fd3m). No extra line corresponding to any other phase within the limit of X - ray detection which is typically 5 % or un-reacted ingredient was detected. The cell edge

parameter a (Å), for the different compositions of spinel ferrite system, $Mg_xMn_{1-x}Fe_2O_4$, has been determined with an accuracy of ± 0.002 Å and is given in Table 1. The lattice constant gradually decreases with increasing $Mg^{2+}$-concentration ($x$) (Table 1). This is due to the replacement of larger $Mn^{2+}$ ion (0.83 Å) by smaller $Mg^{2+}$ with ionic radius of (0.66 Å) in the system [10]. The X-ray density for each composition was calculated, using the relation [10]: $\rho_x = ZM/N_Aa^3$; where Z is the number of molecules per unit cell of the spinel lattice (Z=8), M is the molecular weight of the ferrite composition, $N_A$ is the Avogadro's number and 'a' is the lattice constant. The lattice constant 'a' is found to decrease with $x$, thus, one can expect $\rho_x$ to increase with $x$, but $\rho_x$ is found to decrease with increasing magnesium content ($x$) in the system. This is due to the fact that rate of decrease of 'M' is faster than the rate of decrease of unit cell volume ($a^3$). In order to determine the bulk density ($\rho$) of each specimen in pellet form, precise value of weight measured by electronic balance and the volume measured by using travelling microscope. The $\rho_x$ and $\rho$ decrease with increase in Mg-content ($x$) i.e. the bulk density nearly reflects the same behavior of the theoretical density (Table 1). The porosity (P) of the samples was calculated using the values of X-ray density ($\rho_x$) and bulk density ($\rho$) by the relation; P= (f x 100 %), where, pore fraction (f) is given by the relation, $f = (1-\rho/\rho_x)$. The porosity values for the different compositions are given in Table 1[10]. The average particle size or thickness of the crystal grain diameter (D) for the different samples of Mg-Mn ferrite system with $x$ = 0.0 – 0.5, was calculated from the broadening of the respective high intensity (311) peak using the Debye-Scherrer formula [5]: $D = K\lambda/B.\cos\theta$. Here, $\lambda$ is the wave length of the $CuK_\alpha$ radiation (= 0.15418 nm), constant K (= 0.9) is related both to the crystallite shape and the way in which B and D are defined [10].

TEM images (Figure 3 (a & b) illustrates the nano scale nature of ferrite particles. It can be seen that for $x$ = 0.0 - 0.5 compositions, particles are quite well dispersed and not much agglomeration is present. The particles are perfectly spherical (Figure 3(a) &(b)). The mean particle size for $MnFe_2O_4$ ($x$ = 0.0) is found to be ~ 40 nm, which decreases with increasing Mg- concentration ($x$) and becomes ~ 27 nm for $Mg_{0.5}Mn_{0.5}Fe_2O_4$ ($x$ = 0.5) composition. It is well known that materials with a cubic crystal structure are prone to grow into a spherical shape to minimize the surface tension [10].

The observed decrease in particle size with increasing $Mg^{2+}$ concentration ($x$) ($x$ = 0.0 – 0.5) can be explained as follows. The crystal growth in the solution depends on various parameters, the most important being the molecular concentration of the material approaching the surface of the tiny crystal during the process of growth. Because of the liberation of the latent heat at the surface, the local temperature is normally higher than the solution temperature. The surface temperature affects the local molecular concentration at the crystal surface and hence the crystal growth [10]. It seems that the formation of $Mg_{0.5}Mn_{0.5}Fe_2O_4$ ($x$ = 0.5) is more exothermic as compared to the formation of $MnFe_2O_4$ ($x$ = 0.0). The enthalpy of formation is (–340) KJ/mole for $MnFe_2O_4$ and (–590.7) KJ/mole for $MgFe_2O_4$ [10]. Thus, it is expected that if one introduces magnesium in the system more amount of heat will be liberated, which will increase the surface temperature of the growing crystal, resulting in decrease of the molecular concentration concentration approaching at the crystal surface and hence hindering the crystal growth. The particle size estimated from TEM is slightly greater than the particle size estimated from XRD using Schrrer's formula (Table 1 & 2). This is because X-ray powder diffraction gives the information of crystalline region only and the contribution from the amorphous grain surface does not contribute. On the other hand TEM gives the complete picture of the nanoparticles. By analyzing TEM and XRD, one can have almost complete picture of the particle size, their distribution and morphology.

Five Raman samples of Mg – substituted $MnFe_2O_4$ system have been characterized by Raman spectroscopy i.e. (Figure 4 a – e). Raman spectroscopy can reveal the molecular structure of species present either in a gaseous, liquid or in a solid state provided there are Raman active modes associated with the species under investigation. Mg – Mn ferrite is known to posses cubic spinel structure with unit cell of *fcc* having eight molecules per unit cell and belonging to the Fd3m space group in which Mn, Mg, Fe, O atoms occupy 16d ($D_{3d}$), 8a ($T_d$), 32e($C_{3v}$) Wyckoff sites respectively and give rise to $A_{1g}$, $2A_{2u}$, $E_g$, $2E_u$, $F_{1g}$, $5F_{1u}$, $3T_{2g}$ and $2F_{2u}$, vibrational modes [10]. Five of these modes i.e. $A_{1g}$, $E_g$ and $3T_{2g}$ are Raman active [10] and can be observed under ambient conditions. Here A, E and T notations corresponds to one, two and three dimensional representations where as g denotes the symmetry with respect to the centre of inversion. The five Raman active bands ($A_{1g}$+ $E_g$+ $3T_{2g}$) in the spectra of cubic ferrites involve mainly the

motion of $O^{2-}$ and metal ions. The $A_{1g}$ is the symmetry stretch of tetrahedral $FeO_4$ and appears around 600 – 720 $cm^{-1}$. The $E_g$ is due to symmetry bending of oxygen with respect to Fe and peaks around 250 – 360 $cm^{-1}$. The three $T_{2g}$ modes appear due to anti-symmetric bending/stretching of ligands. The $T_{2g(3)}$ is an anti-symmetric bending of oxygen with respect to Fe and appears around 500 – 590 $cm^{-1}$. $T_{2g(2)}$ is an anti-symmetric stretch of Fe and O and gives rise to a peak around 450 – 520 $cm^{-1}$. $T_{2g(1)}$ is the translational motion of Fe – O reflected close to 180 – 220 $cm^{-1}$ [10].

When the substitution transforms a ferrite from normal to inverse (completely or partially), no change occur in the space group. i.e. Fd3m (in spite the fact that the octahedral sites of the inverse spinel contain two kinds of ions) and therefore, normal and inverse spinel are expected to show the same number of vibrational bands (five active Raman modes). A vibrational frequency is dependent on both, the bond strength (degree of covalence and bond length affect the bond strength) and the reduced mass of the vibrating species. Since the two types of cations are randomly distributed in the spinel lying between normal and inverse, only a change in the frequency in induced [10].

In the present Raman study of $Mg_xMn_{1-x}Fe_2O_4$ ($x$ = 0.0, 0.1, 0.2, 0.4 and 0.5) system, the broadening of Raman bands is observed, this is due to the reduction in the grain size. It is well understood that substitution of Mg in place of Mn in the system leads to reduction in particle size.

In nano crystalline ferrites phonons with q > 0, is involved in scattering process so that broadening and shifting of Raman modes is expected [10]. As shown in Figure 4 (b) and (c), the splitting of $A_{1g}$ mode is observed, this splitting of $A_{1g}$ mode is due to the replacement of heavier $Mn^{2+}$ (54.94 amu) by much lighter $Mg^{2+}$ (24.30 amu). The shifting of $A_{1g}$ mode towards the higher wave number is also observed (blue shift). The change in intensity of vibrational mode as well as shifting, splitting of Raman vibrational modes can be explained on the basis of cation distribution/Particle size reduction. Various Raman modes for the system $Mg_xMn_{1-x}Fe_2O_4$ ($x$ = 0.0, 0.1, 0.2, 0.4 and 0.5) are listed in Table 3.

## CONCLUSIONS

We have successfully synthesized nanocrystalline Mg substituted $MnFe_2O_4$ with chemical formula $Mg_xMn_{1-x}Fe_2O_4$, ($x$ = 0.0 – 0.5) through coprecipitation method, stoichiometry of the samples were confirmed by EDAX and single phase by XRD technique respectively, broadening of the XRD peaks suggests particles with nano scale, further particle size reduces with substitution of $Mg^{2+}$, TEM supports this results. Raman spectral evolution study on nanocrystalline system with general chemical formula $Mg_xMn_{1-x}Fe_2O_4$ ($x$ = 0.0,0.1,0.2,0.4,0.5) clearly demonstrates that the signature of Raman spectra is governed by structural and microstructural parameters, such as site radius, lattice constant, cation distribution, particle size etc, hence one can correlate particle size with Raman scattering.

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**Table 1: Lattice constant (a), X-ray density ($\rho_x$), bulk density ($\rho$), average particle size (D), surface area (S) and equivalent surface free energy ($E_s$) for nano crystalline Mg- Mn ferrites.**

| $Mg^{2+}$ content ($x$) | a (Å) ±0.002Å | $\rho_x$ ($\rho$ (gm/cm³) | P (%) | D (nm) ± 1 nm | S (m²/gm) | $E_s$ (cal/gm) | |
|---|---|---|---|---|---|---|---|
| 0.0 | 8.479 | 5.21 | 4.37 | 16.3 | 38 | 34.36 | 8.14 |
| 0.2 | 8.448 | 4.95 | 4.08 | 17.5 | 33 | 40.83 | 9.68 |
| 0.4 | 8.427 | 4.85 | 3.90 | 19.5 | 28 | 49.58 | 11.75 |
| 0.5 | 8.408 | 4.81 | 3.79 | 21.3 | 25 | 58.66 | 13.90 |

**Table 2 : Average particle size (D) for Mg-Mn ferrite**

| Mg – content ($x$) | D (nm) [XRD] ±1nm | D (nm) [TEM] ±1nm |
|---|---|---|
| 0.0 | 38 | 40 |
| 0.2 | 33 | 36 |
| 0.4 | 28 | 31 |
| 0.5 | 25 | 27 |

**Table 3: Various Raman modes for the system $Mg_xMn_{1-x}Fe_2O_4$ ($x$ = 0.0, 0.1, 0.2,0.4 and 0.5)**

| Mg – content ($x$) | $T_{2g(1)}$ ($cm^{-1}$) | $E_g$ ($cm^{-1}$) | $T_{2g(2)}$ ($cm^{-1}$) | $A_{1g(1)}$ ($cm^{-1}$) | $A_{1g(2)}$ ($cm^{-1}$) | $A_{1g(3)}$ ($cm^{-1}$) | $A_{1g(4)}$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|
| 0.0 | 212.40 | 270.30 | 484.11 | 613.42 | ___ | ___ | ___ |
| 0.1 | 215.96 | 334.92 | 484.05 | 609.89 | 706.52 | 822.44 | 856.24 |
| 0.2 | 208.70 | 331.34 | 484.05 | 606.4 | 699.56 | ___ | ___ |
| 0.4 | 244.95 | 387.76 | 494.06 | 699.66 | ___ | ___ | ___ |
| 0.5 | 230.47 | 325.96 | 491.09 | 637.61 | 696.22 | ___ | ___ |

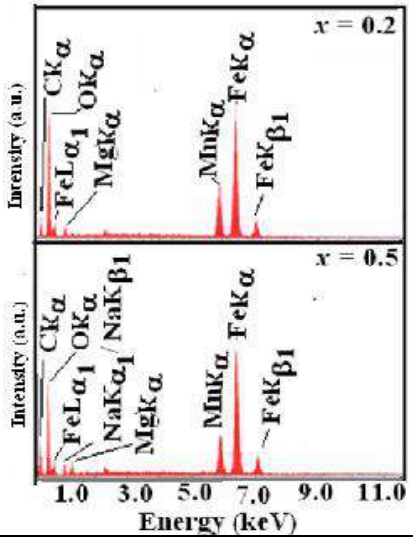


**Figure 1: EDAX spectra of x = 0.2 and 0.5 compositions of Mg-Mn Ferrites**

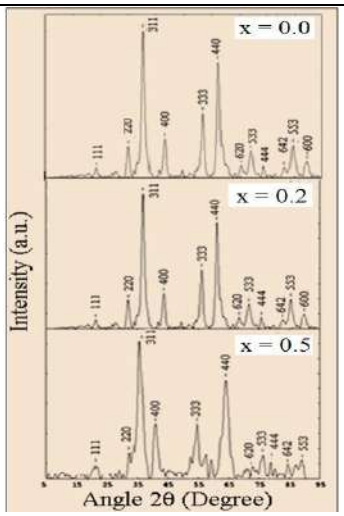


**Figure 2 :X-ray powder diffraction patterns for Mg-Mn spinel ferrite materials recorded at 300 K**

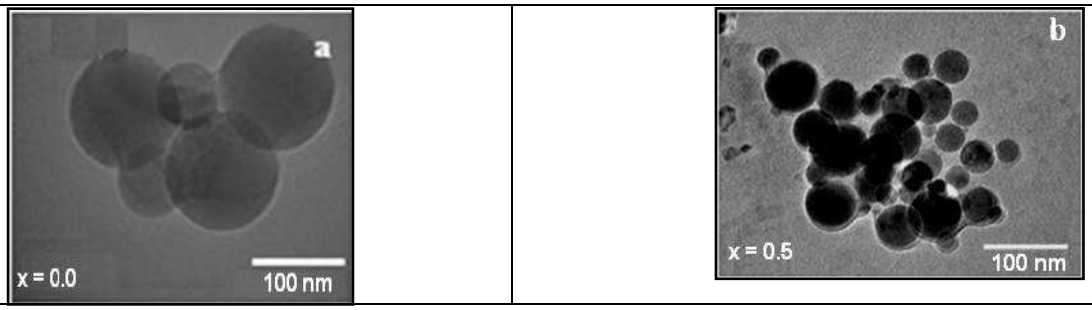


**Figure 3 :TEM images for Mg-Mn ferrites. (a) and (b) – Spherical nano particles of $MnFe_2O_4$ (x = 0.0) and $Mn_{0.5}Mg_{0.5}Fe_2O_4$(x= 0.5)**

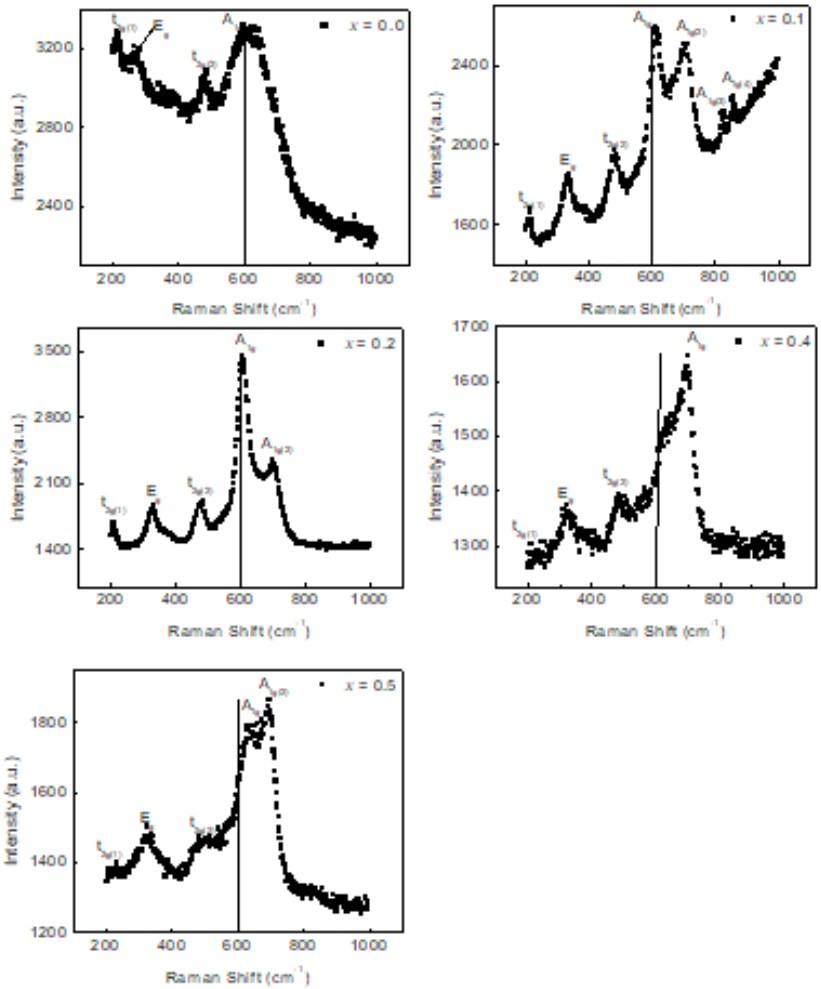


**Figure 4 : Raman spectra for the system $Mg_xMn_{1-x}Fe_2O_4$ (x = 0.0 (a), 0.1(b), 0.2 (c), 0.4 (d) and 0.5 (e))**

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**RESEARCH ARTICLE**

# The Potential Application of R-Phycoerythrin Extract, an Antioxidant-Rich Red Algae (Rhodophyta) Along Gujarat Coast for Potential use in Cosmeceuticals

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## ABSTRACT

Antioxidants are essential for skincare because they protect the skin from oxidative stress and enhance its general health. It is well known that red algae, or Rhodophyta, contain a range of pigments with possible antioxidant qualities. In order to determine whether R-Phycoerythrin, a pigment made from red algae found in collections along Gujarat's coastline, has antioxidant qualities that might be useful in the manufacture of lipstick, this study set out to explore those qualities. R-Phycoerythrin was isolated by breaking down the algal cell wall using a variety of pigment extraction procedures, and it was then purified from crude phycobiliproteins. By means of experimental investigations, antioxidant properties were evaluated. The antioxidant activity of R-Phycoerythrin was shown to be considerable in the results, indicating that it might have applications as a natural antioxidant agent in cosmetics. These results highlight the potential uses of R-Phycoerythrin in lipstick formulations, which provide antioxidant advantages in addition to a bright red hue. Additional investigation in this field might lead to the creation of novel and environmentally friendly cosmetic products.

**Keywords:** Cosmetics, R-Phycoerythrin, Natural ingredient, Red algae, Antioxidant properties

## INTRODUCTION

Researchers are looking into natural sources with potential features since the cosmetics industry is always looking for novel and sustainable components. Specifically after the era of Covid-19 everyone is looking more towards natural products over the chemical one. [1] Red algae (Rhodophyta) are one of these sources that have drawn interest due to the wide range of pigments they contain, such as R-Phycoerythrin, which may have antioxidant properties. [2]Because they reduce oxidative stress and neutralize dangerous free radicals, antioxidants are essential for skincare and cosmetics because they improve the health of the skin as a whole. [3]. Gujarat's shore is home to a large number of red algae, which provide a wealth of bioactive substances, including pigments with variety of colours and possible antioxidant qualities. The pigment known as R-Phycoerythrin, which is commonly present in red algae, has garnered attention due to its intense red hue and its role as an organic antioxidant. [4] R-Phycoerythrin's antioxidant qualities can be extracted and used to provide a sustainable substitute for synthetic antioxidants in cosmetic formulations, especially for lip products where both brilliant colours and skincare benefits are required. [5]. The purpose of this study is to examine the antioxidant qualities of R-Phycoerythrin that was isolated from red algae samples that were gathered along Gujarat's coastline. The study involves breaking down the algal cell wall using several pigment extraction procedures. [6, [7, [8]Then, the crude phycobiliproteins are purified. The antioxidant activity of R-Phycoerythrin will be evaluated in later experiments, shedding light on the substance's possible use in lipstick manufacturing.[9,10,11,12].

This study answers the rising need for sustainable and effective components in cosmetics while also advancing our knowledge of natural pigments made from red algae by clarifying the antioxidant potential of R-Phycoerythrin. [13,14,15] In addition, the investigation of R-Phycoerythrin as a multipurpose ingredient in lipstick formulas highlights the multidisciplinary character of cosmetic research, incorporating elements of biology, chemistry, and skincare. [16,17]. In summary, by investigating the antioxidant qualities of R-Phycoerythrin from red algae along the Gujarat coastline, this work aims to close the gap between the fields of natural product chemistry, cosmetics, and environmental sustainability. The results of this study might guide the creation of innovative, eco-friendly cosmetic formulas. [8,18].

## MATERIALS AND METHODS

**Collecting and Preparing Samples**

Samples of red algae (Rhodophyta) were taken from the intertidal zones throughout the Gujarat coast, with a focus on regions with high algal populations. In order to assure representative collection across seasonal fluctuations, sampling was done throughout during the months of January to April from (20.7568750, 70.6589300) site. [19] collected Samples were transported to the laboratory from the intertidal zones along the Gujarat coastline. The biomass was collected and prepared upon arrival in order to ensure homogeneity and eliminate impurities in advance of the extraction process. [20]

**Preparation of Algal Biomass for the pigment extraction**

Liquid nitrogen was applied to the gathered algal biomass in order to break down cell structures and accelerate up the extraction procedure. After that, the biomass was homogenized at a pH of 7.2 ± 0.2 using ice-cold 0.1 M phosphate buffer. Temperature of the pigment extraction system has been maintained at 4 °C throughout the process. reason to maintain the temperature is to eliminate the chances to lose the pigment. The algal biomass to phosphate buffer ratio was kept constant at 1:10. Phycobiliprotein was isolated by breaking down the algal cell wall using various kinds of pigment extraction procedures. The following techniques were applied.

**Pigment extraction protocol**

To cause additional harm to the algal cells and extract intracellular pigments, ultrasonic sonication was used. For the sonication procedure, a 40 kHz ultrasonic bath sonicator device running at 200 W power was used for 30 minutes, the

algal biomass suspended in phosphate buffer was subjected to sonication treatment. Furthermore, without sonication treatment, a consistent standard was maintained for thirty minutes. In order to prevent heat-induced pigment degradation, the sonication procedure was carried out with the system temperature kept at 4 °C. The crude extract was centrifuged at 12,000 g for 30 minutes at 4 °C after being sonicated. The separation of insoluble materials and cellular debris was made easier by this centrifugation process, which produced a supernatant that included the crude pigment extract. After being collected, the supernatant was utilized for further purification and analysis. The pigment concentration and purity of the resultant crude extract were assessed by spectroscopic methods, such as UV-Vis spectrophotometry. To describe the extracted pigments, absorbance measurements were taken at the proper wavelengths. The phycobiliproteins were quantified by following the method as described by Bennett & Bogorad (1973). [21,22].

The formula followed are given below:
Phycocyanin (PC) (mg mL−1) = [A615−0.474 (A652)] / 5.34
Allophycocyanin (APC) (mg mL−1) = [A652−0.208 (A615)] / 5.09
Phycoerythrin (PE) (mg mL−1) = [A562−2.41 (PC) −0.849 (APC)] / 9.62

**Dialysis:**
Ammonium sulphate was used to precipitate phycoerythrin from the crude extract after the crude pigment was extracted. After ammonium sulphate was saturated, the crude extract was gradually added while being stirred constantly until the required saturation level was obtained. The mixture was then allowed to precipitate phycoerythrin by being incubated at 4 °C for a whole night. Next, by centrifuging at 12,000 g for 30 minutes at 4 °C, the precipitated phycoerythrin was recovered. [9]. Dialysis was carried out overnight on the precipitate that was produced containing phycoerythrin with the objective to flush out excess ammonium sulphate and other small molecular weight contaminants. With mild agitation, dialysis was carried out against phosphate buffer using dialysis membrane to guarantee effective impurity removal while maintaining the structure of phycoerythrin.

**Column chromatography**
Chromatography was used to further purify the phycoerythrin from the crude pigment. The column chromatography approach was used because of the particular features of phycoerythrin. The crude extract of dialyzed precipitate was put onto the chromatography column, which was equilibrated with 0.1 M phosphate buffer (pH 7.2). Elution was performed using a gradient to separate and purify the phycoerythrin from the other components present in the crude extract. [23]. The pure phycoerythrin fractions produced by chromatography were dialyzed overnight to eliminate any remaining salts or buffer components. Dialysis was done using dialysis membrane and phosphate buffer (pH 6.8). the setup was kept at 4˚C under moderate mixing. The buffer was changed every 8 hours to guarantee the end product's purity and stability.

**Analysis of Antioxidant Activities**
The antioxidant activity of pure R-phycoerythrin was assessed utilizing a variety of in vitro tests. The tests comprised DPPH, Super Oxide, Nitric oxide, $H_2O_2$, and FARP to assess free radical scavenging activity, reducing power, and antioxidant capacity. Absorbance and other relevant measures were taken using a UV-VIS Spectrophotometer, and antioxidant activity was computed using standard curves or reference compounds. [24,25,26,27]

## RESULTS

**Extracting and Purifying Pigments**
The first crude extract produced by the extraction of R-Phycoerythrin (R-PE) from red algae gathered along the Gujarat coastline had a vivid pink hue. Optimizing the Duration of Sonication: After 20 minutes of ultrasonic sonication, the maximum pigment production was seen, suggesting ideal cell rupture without any pigment loss.

**Spectroscopic Examination:** The R-PE-specific strong absorption peak at 565 nm was evident in the crude extract. The precipitation of ammonium sulphate, which precipitated the phycoerythrin preferentially, enhanced the quality of the crude extract even further. Ammonium Sulphate Precipitation: The crude extract was progressively added to a saturated solution of ammonium sulphate until 60% saturation was reached. This was followed by a two-hour incubation period at 4°C throughout the purification process. Centrifugation was used to collect the precipitate for 30 minutes at 4°C at 12,000 g.

**Dialysis:** To remove any remaining ammonium sulphate, the precipitate was dialyzed overnight against 0.1 M phosphate buffer, pH 7.2, using a 10 kDa cut-off dialysis membrane.
**Chromatography Purification**: R-PE-containing fractions were distinguished by their absorbance at 565 nm using size exclusion chromatography. Any leftover salts were guaranteed to be removed by further dialysis of the purified fractions. R-phycoerythrin pigment's biological characteristics as its antioxidant activity has been shown in the chart form for the better illustration.
**Colour Stability:** Under ambient storage settings for three months, the colour of R-PE did not change.
Antioxidant Efficacy: When DPPH test was used to assess antioxidant activity, R-PE performed better than formulations lacking R-PE.

## DISCUSSION

R-Phycoerythrin, which has strong antioxidant qualities, can be extracted and purified from red algae with success. Strong free radical scavenging and reduction abilities were demonstrated by the R-PE that was produced using an improved sonication technique and subsequent purification processes. As demonstrated by the DPPH, ABTS, and FRAP tests, R-PE has antioxidant activity, which points to its possible application as a natural addition in cosmetics. R-PE added further antioxidant advantages to lipstick formulas in addition to providing bright, reliable colour that can help shield the lips from environmental damage and oxidative stress.

The study emphasizes R-PE's dual use in lipstick formulations as an antioxidant and colorant. This is in line with the growing desire of consumers for natural and multipurpose cosmetic components. R-PE is a desirable substitute for synthetic colours and antioxidants that are frequently used in cosmetics due to its excellent purity and durability.

## CONCLUSION

This study successfully demonstrated the potent antioxidant properties of R-Phycoerythrin extracted from red algae along the Gujarat coastline and its potential future application in lipstick production. The natural pigment not only provided vibrant colour but also offered protective antioxidant benefits, making it a promising ingredient for future cosmetic formulations. More research and development are warranted to optimize its use and confirm its benefits in commercial products. A preliminary application research was conducted to determine the possible use of pure phycoerythrin in cosmetics, specifically lipstick manufacture. The optimization of the extraction and purification procedure for large-scale manufacturing and the assessment of R-PE stability in cosmetic formulations are among the study's shortcomings. Long-term investigations on the safety and effectiveness of R-PE in topical applications, as well as its synergistic effects with other cosmetic components, should be the focus of future study.

**Conflict of Interest**
There is no conflict of interest by authors.

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**Figure 1: algal sample collection site**

**Figure 2: algal sample obtained from coastline**

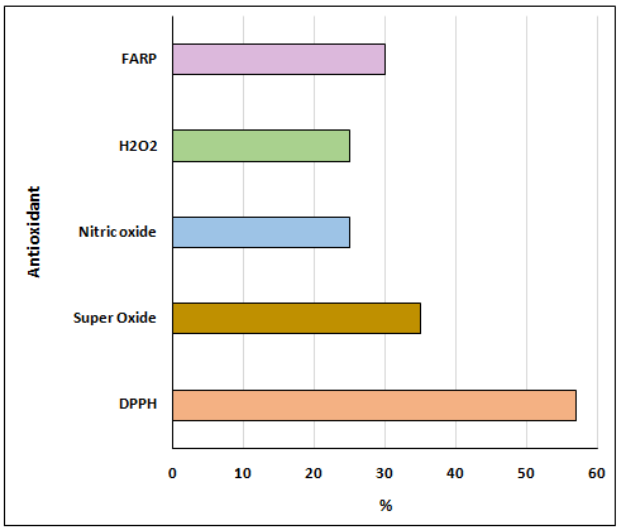


**Figure3: Biological properties of R-phycoerythrin pigment (Antioxidant activity)**

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**RESEARCH ARTICLE**

# Dynamic Power Optimization in Multi-Tenant Cloud Systems and Edge Computing

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## ABSTRACT

As cloud computing and edge computing continue to advance, optimizing power consumption in multi-tenant environments has become increasingly crucial. This paper presents a comprehensive approach to dynamic power optimization in multi-tenant cloud systems integrated with edge computing. We propose a framework that addresses the unique challenges of managing power across both centralized cloud data centres and distributed edge nodes, where multiple clients share resources with varying demands. Our framework leverages real-time monitoring and adaptive algorithms to balance power efficiency and performance. Key components include dynamic workload distribution, energy-efficient resource allocation, and the application of Dynamic Voltage and Frequency Scaling (DVFS) techniques. By incorporating predictive modelling, our approach forecasts workload fluctuations, enabling proactive adjustments to power settings and resource allocations. We investigate the impact of edge computing on power optimization, highlighting how local processing at edge nodes reduces the burden on central cloud infrastructure, thus lowering overall energy consumption and latency. Our methodology also involves localized power management strategies at the edge, including resource offloading and power-saving techniques. Through extensive simulations and case studies, we demonstrate that our framework significantly improves energy efficiency while maintaining high performance and service quality. The results indicate that dynamic power optimization not only reduces operational costs but also supports sustainability goals by lowering the carbon footprint of cloud and edge computing environments. This paper provides valuable insights and recommendations for future research and practical

implementations in the field of power optimization for multi-tenant cloud systems integrated with edge computing.

**Keywords:** Cloud computing, Power management, Multi-tenant cloud systems, Dynamic power optimization, Real-time monitoring.

## INTRODUCTION

The rapid growth of cloud computing has transformed how businesses and organizations deploy and manage their IT infrastructure. In particular, multi-tenant cloud systems, where multiple clients share the same physical resources, have become a prevalent model due to their scalability, flexibility, and cost-effectiveness. However, this shared infrastructure introduces unique challenges, particularly in terms of power management. As data centres expand to support the increasing demand for cloud services, the energy required to maintain these systems has grown significantly, contributing to operational costs and environmental impact. Efficient power management in multi-tenant environments is not only a matter of reducing energy consumption but also ensuring that system performance and service quality are not compromised. Traditional static power management techniques often fail to adapt to the dynamic nature of cloud workloads, which fluctuate in real-time based on user demands and operational needs. Therefore, there is a critical need for more advanced power optimization strategies that can respond dynamically to these changes. This paper addresses this challenge by presenting a novel framework designed to optimize power consumption in multi-tenant cloud systems. By leveraging real-time monitoring, adaptive algorithms, and predictive modelling, our approach aims to balance energy efficiency with system responsiveness. We explore key aspects of power optimization, including dynamic workload balancing, energy-efficient resource allocation, and the role of virtualization technologies. Additionally, we evaluate the effectiveness of different strategies through extensive simulations and case studies, providing actionable insights for improving energy efficiency in cloud data centres. Popular Section 5, we discuss the fallouts and insights gained from the case studies, and lastly, trendy Section 6, we arrange with recommendations for future research directions in dynamic power management for multi-tenant cloud systems.

**Cloud Computing**

Cloud computing allows users to access and use computing resources remotely via the internet, rather than relying on local servers or personal devices. This model offers flexibility, scalability, and cost-effectiveness, as resources can be adjusted based on demand. This allows users to access and utilize these properties on-demand, lacking the requirement to manage physical organization or hardware. Fog calculation provides some key rewards, with scalability, flexibility, cost savings, and ease of maintenance, making it a vital technology for businesses, organizations, and individuals alike.

**Infrastructure as a Service (IaaS)**: Provides virtualized computation properties completed the cyberspace, such as simulated technologies, storing and networks. Users can scale infrastructure built on their desires, reducing the essential to invest in carnal hardware.

**Platform as a Service (PaaS)**: PaaS is a cloud computing model that provides developers with a platform to create, test, and deploy applications without worrying about the underlying hardware and software infrastructure. It typically includes tools for application development, database management, middleware, and other services that streamline the development process.

**Software as a Service (SaaS)**: Offers software system requests via cloud, classically accessed through a web browser. SaaS removes the requirement for users to install or keep software locally, providing regular updates and scalability.

**Power Management**

Power management refers to the process of efficiently controlling and optimizing the consumption of electrical energy in systems and devices. In the context of computing, especially cloud computing and data centres, power management is crucial for reducing operational costs, improving system reliability, and minimizing environmental impact. The importance of power management has increased significantly as modern data centres and computing infrastructures grow larger and more complex. These systems must support massive computational loads while minimizing energy consumption to meet sustainability goals and reduce electricity costs. Key aspects of power management include:

**Dynamic Power Allocation**: Adjusting power usage in real-time based on system demand and workload. This ensures that resources are allocated efficiently to meet performance requirements without wasting energy.

**Energy-Efficient Resource Allocation**: Optimizing the distribution of computing tasks across physical and virtual resources to reduce the total energy consumption while maintaining system performance and service quality.

**Workload Consolidation**: Consolidating tasks on fewer physical machines when system utilization is low, allowing idle machines to be powered down or put into low-power states, thereby reducing energy use.

**Virtualization and Power Optimization**: Using virtualization technologies to create multiple virtual machines (VMs) on fewer physical servers, allowing better resource utilization and lower power consumption. Virtualization also enables dynamic scaling and migration of workloads, which can help distribute power load more efficiently across data centres.

**Cooling and Thermal Management**: Power consumption is closely linked to heat generation, so effective cooling solutions are essential for maintaining optimal temperatures in data centres. Advanced cooling techniques, such as liquid cooling and hot aisle containment, both techniques can significantly lower energy consumption, reduce operational costs, and enhance overall cooling performance.

**Power Usage Effectiveness (PUE)**: A metrical used to measure energy efficiency to data centres. It is the ratio of total energy used by the data centre (with power for IT equipment, cooling, and other infrastructure) to the energy used solely by the IT equipment. The goal is to minor the PUE to make data centre more energy-efficient.

**Multi-Tenant Cloud Systems**

Multi-tenant cloud systems refer to cloud environments where multiple clients (tenants) portion the same physical or virtual structure while sustaining isolation and privacy between their respective data, applications. This architecture allows service providers to optimize resource utilization by hosting multiple users on shared hardware, reducing costs and improving efficiency. Each tenant operates independently, unaware of the other tenants, while sharing the underlying resources like storage, CPU, and memory. Multi-tenant cloud systems are commonly used in public cloud environments, where cloud service providers offer scalable and flexible computing resources to various users. The tenants can range from individuals to enterprises, each with different applications, workloads, and performance requirements.

**Resource Sharing:** Multiple tenants share the same physical infrastructure (servers, storage, and networking) but are isolated at the software level to ensure security, privacy, and performance segregation. This allows efficient utilization of resources and reduces operational costs for both providers and users.

**Scalability**: Multi-tenant systems are designed to scale easily by allocating resources dynamically based on tenant demand. As tenant workloads fluctuate, the system can adjust to provide additional resources when needed or release them when no longer required.

**Isolation and Security**: Even though tenants share physical resources, they operate in isolated environments where one tenant's data or activities are protected from other tenants. Various techniques such as virtualization, encryption, and network segmentation are employed to ensure data privacy and security.

**Cost Efficiency**: Since multiple tenants share the same resources, the cost of infrastructure is spread across users, making it more economical for both the cloud provider and its customers. This cost efficiency is a key benefit of cloud adoption, particularly for small and medium-sized businesses.

**Customization and Flexibility**: Multi-tenant cloud systems allow each tenant to configure their virtual environment to suit their specific needs. They can manage applications, storage, and networking independently without affecting other tenants.

**Dynamic Resource Allocation**: Resources in multi-tenant environments are dynamically allocated based on the tenants' needs. This means that tenants can rule up or down their incomes (e.g., CPU, RAM, storage) as their workloads require, ensuring optimal performance and energy efficiency.

**Management of Diverse Workloads**: In a multi-tenant cloud, different tenants may run diverse workloads—ranging from compute-intensive applications to storage-heavy tasks. The system must efficiently manage these heterogeneous workloads to ensure that each tenant receives the necessary resources without impacting the performance of others.

**Dynamic Power Optimization**

Dynamic power optimization refers to the process of continuously adjusting power consumption in real-time built on the current load and system conditions to realize optimal energy efficiency without compromising performance. This technique is particularly important in computing environments such as cloud data centres, where energy consumption can be a major operational cost. Dynamic power optimization aims to reduce the power used by computing resources like servers, storage systems, and networking equipment while maintaining the quality of service (QoS) for end users. In cloud computing, dynamic power optimization is especially relevant for multi-tenant systems where multiple clients share resources and workloads can vary significantly. The need for real-time adjustments to power usage arises due to fluctuating demand, making static power management strategies inefficient in these dynamic environments. Key components of dynamic power optimization include:

**Real-Time Monitoring**: The system continuously monitors resource utilization, workload characteristics, and power consumption across the infrastructure. This data is critical for making informed decisions about how to adjust power usage to meet changing conditions.

**Adaptive Algorithms**: These algorithms automatically adjust power levels in response to real-time data. They analyze the system's current state, predict future workload demands, and make adjustments such as scaling resources up or down, changing processor speeds, or turning off idle components. Adaptive algorithms ensure that power is not wasted on underutilized resources, and that system performance is maintained.

**Dynamic Voltage and Frequency Scaling (DVFS)**: DVFS is a commonly used system in dynamic control optimization. It adjusts the voltage and frequency of processors based on workload demands. Lowering the voltage and frequency reduces power consumption during periods of low activity, while increasing them ensures performance during high-demand periods.

**Workload Balancing**: Dynamic power optimization often involves distributing workloads across available resources in an energy-efficient manner. By balancing workloads, the system can reduce the number of active servers or shift workloads to more energy-efficient machines, allowing some servers to enter low-power states.

**Energy-Efficient Resource Allocation**: Resources such as CPU, memory, and storage are allocated based on the actual needs of workloads, minimizing energy waste. For example, cloud providers may consolidate multiple virtual machines (VMs) onto fewer physical servers during periods of low activity, allowing unused servers to be powered down.

**Predictive Modelling**: Machine learning and predictive models are often used to forecast future workload patterns and adjust power settings in advance. By predicting demand, the system can proactively manage resources and prevent performance degradation due to sudden spikes in activity.

**Virtualization and Power Efficiency**: Virtualization technologies enable extra efficient use of computer hardware by allowing many virtual machines to run on a single physical server.
Dynamic power optimization can leverage virtualization by dynamically migrating VMs across servers to balance load and minimize power consumption.

**Management**: In addition to optimizing power usage, dynamic power optimization systems often include thermal management techniques. By monitoring temperature, the system can adjust workloads or fan speeds to keep hardware at an optimal operating temperature, further reducing the need for excessive cooling.

**Real-Time Monitoring**
Real-time monitoring refers to the nonstop tracking and analysis of system performance, resource usage, and operational metrics as they occur, without delay. In computing environments, particularly cloud computing and data centres, real-time monitoring is essential for ensuring that resources are used efficiently, performance levels are maintained, and issues are quickly identified and resolved. By observing live data, systems can make immediate adjustments to optimize performance, power consumption, security, and overall system health. In the context of cloud computing, real-time monitoring enables dynamic decision-making, such as power optimization, resource allocation, and workload balancing, making it a critical component of modern infrastructure management. Key aspects of real-time monitoring include:

**Continuous Data Collection**: Real-time monitoring involves the constant collection of data from various system components, such as CPU usage, memory ingesting, road traffic, disk I/O, power usage. This data is transmitted to a central monitoring structure or dashboard further breakdown and visualization.

**Immediate Analysis and Alerts**: Data collected in real-time is analyzed immediately to detect anomalies, performance bottlenecks, or resource overutilization. Alerts and notifications are generated when thresholds are breached, enabling administrators to take quick action to resolve potential issues before they affect system performance or availability.

**Dynamic Resource Allocation**: Real-time monitoring plays a key role in dynamic resource management. As workloads fluctuate, real-time insights enable the system to allocate or deal locate resources (e.g., CPU, memory, storage) based on the actual demand, optimizing performance and energy efficiency.

**Power Consumption Monitoring**: For dynamic power optimization, real-time monitoring of power consumption is critical. By tracking how much power each server or component is consuming at any given time, the system can make adjustments, such as scaling down power to underutilized resources or consolidating workloads to reduce energy use.
**Workload Performance**: Monitoring the performance of running applications and workloads is vital to ensuring they meet the required quality of service (QoS) levels. Real-time monitoring can track factors like latency, response times, and throughput, ensuring that any dips in performance are addressed quickly.

**Predictive Analytics**: Real-time monitoring provides the raw data necessary for predictive modelling and analytics. With enough historical and live data, predictive algorithms can forecast future resource demand, potential failures, or performance bottlenecks. This allows systems to make proactive adjustments before problems arise.

**Health and Security Monitoring**: In addition to performance metrics, real-time monitoring tracks system health, such as hardware temperatures, fan speeds, and disk integrity. It also plays a key role in security by nonstop monitoring network traffic, user behavior, system logs for signs of breaches or unauthorized access.

**Visualization and Dashboards**: Real-time monitoring systems typically feature dashboards that display key metrics in an easily digestible format. Administrators can view live data, spot trends, and drill down into specific components to troubleshoot issues.

**Automation Integration**: Real-time monitoring systems often integrate with automation tools to implement automated responses to detected issues. For example, if a server becomes over utilized, the system can automatically provision additional resources, or if an application experiences a failure, the system can trigger a failover.

## METHODOLOGY

**Problem Definition and Objectives**

**Problem Statement**: Clearly define the problem you aim to solve—optimizing power management in multi-tenant cloud systems while maintaining performance.

**Objectives**: Specify the goals of the study, such as reducing energy consumption, ensuring system responsiveness, improving resource allocation, and evaluating performance through simulations.

**Simulation Environment Setup**

**Cloud Architecture Model**: Develop or use an existing cloud infrastructure model that simulates a multi-tenant environment. This model should represent the core components of a cloud data centre, including servers, virtual machines (VMs), hypervisors, and networking equipment.

**Workload Generator**: Use or create a workload generator that simulates various user demands, reflecting the dynamic nature of cloud workloads. These workloads can vary in intensity, duration, and resource needs.

**Proposed Framework**

**Real-Time Monitoring**: Implement mechanisms to monitor power consumption, resource utilization, and system performance in real time. Use sensors or software tools that gather data on CPU, memory, and network usage across virtualized environments.

**Adaptive Algorithms**: Introduce adaptive algorithms that adjust power management strategies dynamically. These algorithms can include techniques like dynamic voltage and frequency scaling (DVFS), power capping, and live VM migration to balance workload distribution.

**Predictive Modelling**: Incorporate predictive models to anticipate future workload demands and power consumption patterns. These models can use machine learning techniques to forecast load fluctuations and pre-emptively adjust system settings.

**Simulation and Evaluation Methodology**

**Metrics**: Define key performance indicators (KPIs) for evaluation, such as energy consumption (kWh), system performance (response time, throughput), and Quality of Service (QoS) parameters (latency, availability).

**Scenarios**: Simulate various real-world scenarios, including peak workloads, average loads, and underutilization, to assess the robustness of your power management strategies.

**Baseline Comparison**: Compare your proposed methods against traditional static power management techniques to showcase the improvements in energy efficiency and system responsiveness.

**Tools**: Use simulation platforms such as Cloud Sim, Green Cloud, or custom-built tools to estimate the presentation of the future framework in different cloud environments.

**Data Analysis**

**Energy Consumption Analysis**: Analyze the power consumption data and compare the results across different strategies, including the proposed adaptive methods and traditional approaches.

**System Performance Evaluation**: Evaluate the system's responsiveness and performance under varying workloads, identifying any trade-offs between energy efficiency and service quality.

**Statistical Validation**: Use statistical methods to validate the significance of your findings, ensuring that the observed improvements in energy efficiency and performance are not random.

**Benefits**

**Reduced Energy Consumption**: By optimizing power usage in real-time, data centres and cloud providers can meaningly lower their energy consumption, resultant in cost reserves and reduced environmental impact.

**Maintained Performance**: Adaptive power management ensures that the system meets performance requirements by allocating resources where they are needed most while avoiding power waste.

**Improved Resource Utilization**: By dynamically adjusting resource allocations and power settings, systems can maximize resource efficiency, using fewer physical servers to meet demand.

**Immediate Issue Detection**: Monitoring data in real-time permits for instant papers of presentation issues, failures, security breaches, reducing downtime and improving reliability.

**Optimized Resource Usage**: By tracking system utilization in real-time, resources can be dynamically adjusted to meet current demand, avoiding over provisioning and underutilization.

**Improved Power Efficiency**: Real-time insights into power usage enable dynamic power management, reducing energy consumption and operational costs.

**Enhanced System Health and Security**: Constant monitoring of hardware health and security metrics ensures that potential threats or hardware failures are identified before they escalate.

**Actionable Insights for Decision-Making**: The live data provided by real-time monitoring is crucial for data-driven decision-making, particularly in complex, dynamic environments like cloud computing.

**Challenges**

**Complexity**: Implementing dynamic power optimization requires sophisticated monitoring and control systems capable of making rapid adjustments without negatively affecting performance.

**Overhead**: The algorithms and monitoring systems required for dynamic power optimization can introduce computational overhead, which itself consumes power.

**Workload Variability**: Predicting future workloads and adjusting power settings accordingly can be challenging, especially in environments with highly unpredictable or spiky workloads.

**Performance Variability**: With multiple tenants sharing the same infrastructure, performance can fluctuate depending on resource contention and system load.

**Security and Privacy**: Ensuring strict isolation between tenants to prevent data breaches or cross-tenant attacks is crucial in multi-tenant environments.

**Power Management**: Efficiently managing power across diverse tenants with varying workloads is a complex challenge, as over- or under-provisioning resources can lead to inefficiencies and increased operational costs.

**Data Overload**: Real-time monitoring generates large volumes of data, which can overwhelm systems if not managed properly. Effective filtering and prioritization of critical metrics are essential.

**Latency in Response**: Although real-time monitoring aims for instant response, the system's ability to react quickly depends on how efficiently alerts are processed and acted upon.

**Implementation Complexity**: Setting up a robust real-time monitoring system can be complex, requiring the integration of various tools, sensors, and automation systems.

## CONCLUSION

In conclusion, dynamic power optimization is a crucial strategy for improving energy efficiency in cloud computing and data centre environments. By leveraging real-time data, adaptive algorithms, and advanced techniques like DVFS and workload balancing, it helps minimize energy use while maintaining system responsiveness and performance. This leads to significant cost savings, enhanced sustainability, and more efficient cloud infrastructure management. Multi-tenant cloud systems offer scalability, price savings, flexibility, making them attractive option cloud service providers and clients alike. However, challenges such as resource allocation, security, and performance must be carefully managed to ensure a balanced, efficient, and secure cloud environment. Real-time monitoring is a foundational technology for managing modern computing environments, including cloud data centres. By providing continuous visibility into system performance, power usage, and security, real-time monitoring enables dynamic, data-driven optimization of resources, contributing to improved efficiency, lower costs, and enhanced reliability.

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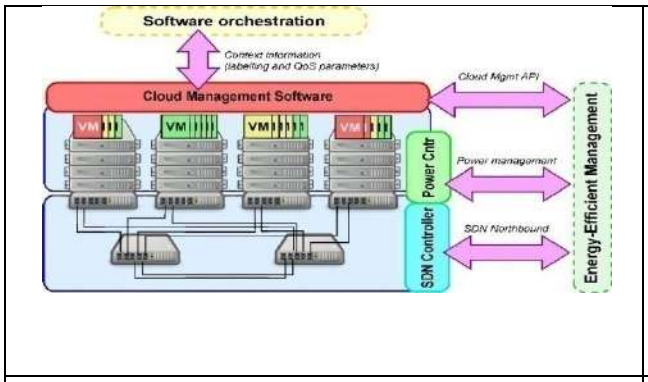


**Fig 1 : Framework for Energy-Efficient Infrastructure**

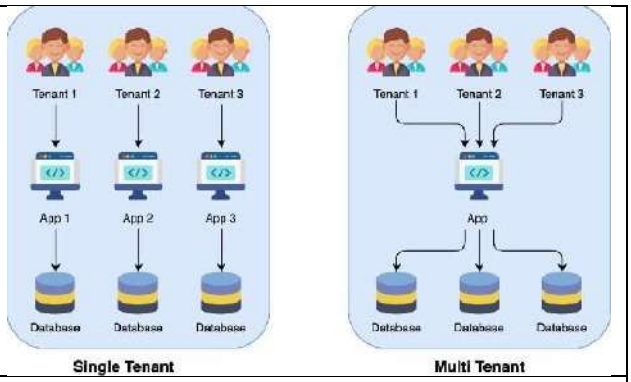


**Fig 2 : Tenant-Sharing**

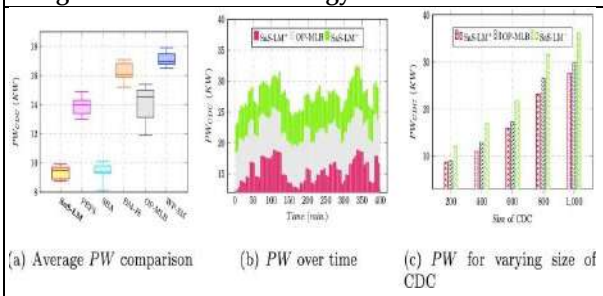


**Fig 3: Power Utilization (1)**

| Mode | Core | Drowsy | Dull |
|---|---|---|---|
| Baseline | 1.08V 125MHz | 1.08V 125MHz | 1.08V 125MHz |
| Slow | 1.08V 125MHz | 1.08V 125MHz | 0.9V 66MHz |
| Standby | 0.0V | 1.08V 125MHz | 0.0V |

**Fig 4: DVFS**

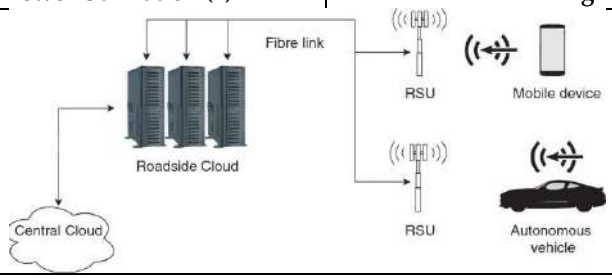


**Fig 5: Edge-Based DVFS**

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**RESEARCH ARTICLE**

# A Real-Time System for Face Mask Detection and Ensuring Social Distancing Compliance Based on Deep Learning

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## ABSTRACT

The emergence of the COVID-19 virus and its subsequent global spread have precipitated an unprecedented public health crisis, claiming over more than five million lives worldwide, including a staggering over half a million 533570 to be exact lives in India. Urgent measures were essential to mitigate further devastation. While vaccines offer hope, the importance of sustained preventive strategies, such as face mask usage and social distancing, cannot be under estimated. In response to these challenges, this study introduces a real-time surveillance system designed to analyze video feeds to assess adherence to the Face Mask and Social distancing guidelines. This work leverages YOLOv5 object detection framework to identify human subjects within the video stream. Subsequently, a Stacked Res-Net classifier is employed to determine whether these detected individuals are wearing face masks. Additionally, the system extends its capabilities beyond face mask detection by utilizing DBSCAN to identify instances of proximity among detected individuals, thereby monitoring adherence to social distancing norms. Through the integration of these advanced technologies, the proposed system offers a comprehensive approach to reducing the risk of virus transmission. In an era where technology is reshaping various aspects of human existence, this research stands at the intersection of innovation and public health. Beyond its immediate implications, it highlights the potential of technology in safeguarding society during crises, emphasizing the significance of innovation in times of adversity, where creative solutions can emerge from the synergy of artificial intelligence and public well-being. As societies worldwide chart the path to recovery, this real-time surveillance system presents a proactive strategy to complement vaccination efforts. Through continuous monitoring and enforcement of preventive measures, we move closer to a safer, healthier future.

**Keywords:** Face Mask, Social Distancing, Real-Time surveillance, YOLOv5.

# INTRODUCTION

Deep mastering is a subset of machine learning (ML), that's itself a subset of artificial intelligence (AI) and is centered on enhancing that technique of having machines study new matters. With rule based totally AI and ML a data scientist determines the rules and records set functions to encompass in fashions, which drives how the ones fashions perform. With deep learning, the data scientist feeds raw data into an algorithm. The system then analyzes that data, without specific rules or features preprogrammed into it. Once the system makes its predictions, they are checked against a separate set of data for accuracy. The level of accuracy of these predictions—or lack thereof—then informs the next set of predictions the system makes.

The "deep" in deep mastering refers to the various layers the neural community accumulates through the years, with performance improving as the community receives deeper. each level of the network techniques its enter records in a specific way, which then informs the next layer. So the output from one layer will become the input for the subsequent. Training deep gaining knowledge of networks is time consuming and requires huge quantities of information to be ingested and examined towards as the gadget step by step refines its model. Neural nets were round because the Fifties, but best in current years have both computational energy and data garage abilities superior to the factor in which deep studying algorithms can be used to create thrilling new technology. For example, deep learning neural networks that have made it possible for computers to carry out tasks like speech recognition, computer vision, bioinformatics, and medical image analysis.

**Application of Deep Learning**

Deep learning uses big neural networks with many layers of processing, taking advantage of advances in computing capacity and improved education techniques to learn complex equations and formulas in massive amounts of data. The most common data packages consist of image and speech recognition.

The first case for coronavirus (COVID-19) was reported for in December 2019 in the capital of Hubei Province, Wuhan, China. After a couple of months, the infection spread worldwide in the year 2020. And very quickly, in May 2020, the World Health Organization (WHO) declared the circumstance as a pandemic for the whole world [11]. The insights by WHO on October 8, 2020 affirmed 36million infected individuals and a startling number greater than one million life losses over couple of hundred nations. Also, with the developing pattern of infected people, there was still no known or quick cure for the infection. While scientists, clinical experts were continuously endeavoring to find proper solutions or cure for the dangerous disease entity, no solution had been found till then. However, some vaccines were under trial.

Since there is no permanent cure of this disease and the infection spreads fast and is a threat to humankind, we have to take care for it ourselves. It was seen that social distancing was on of th best methods to reduce transmission. One of the best modes to reduce the spread of new cases of COVID-19, was by keeping some form of social isolation.

Social isolation or distancing implies making changes in your daily routine or normal activities in such a way that on limits close contact with others. This can be done by

1. Being away from crowded places and avoiding get-togethers.
2. Staying away from common gatherings and greetings, like handshakes.
3. Restricting the contact with peoples who are potentially at higher risk (too old, reduced immunity, too young, low weight, persons with chronic illness).
4. Maintaining a distance roughly up to 1 meter between individuals is considered to be the bare minimum to reduce the transmission to a great extent.
5. The most important among all was wearing of masks, forming a physical barrier to the transmission.

In this paper, the YoloV5 was applied to perform the CNN to recognize whether a person wears a face mask properly, wears a face mask incorrectly, or does not wear a face mask. The experimental results for face mask detection obtained from the deep learning models.

**Techniques Used**

***YOLOv5***

YOLO stands for You Only Look Once and is quite possibly the most famous item indicator convolutional neural organizations (CNNs). It was Joseph Redmon et al. who presented their paper first based on YOLO in 2015[12], bringing about the variations that were dispersed by them in the next two years and by Alexey Bochkovskiy in the year 2020[13].

**Previous versions of YOLO are –YOLO**

It utilizes Darknet structure which is prepared on ImageNet-1000 dataset. This functions as a reference yet has numerous limitations in view of it the utilization of the YOLO v1 is concerned. It couldn't discover little items on the off chance that they are showed up as a group. This engineering had trouble in discovering of items if the picture w as of different measurements not quite the same as the prepared picture.

**Demerits of YOLO**

- YOLO can distinguish just 49 objects.
- Generally high localization error.

**YOLOv2**

Another kind of YOLO that is named YOLO9000, has been proposed by Joseph Redmon and Ali Farhadi towards the end of 2016[14]. The significant upgrades of this variety are better, quicker and can integrate with faster R-CNN program. The major changes with respect to YOLO v1 are: Batch Normalization, High-Resolution Classifier, use of Anchor Boxes for Bounding Boxes and Multi-Scale Training.

**YOLOv3**

The past variant has been steadily improved, that is as of now called YOLO v3. As many object detection algorithms are there for some time now, the question is, how precise and rapidly are the objects detected and distinguished. YOLO v3 has all we require for object location continuously with precise classification of the objects. YOLO v3 uses a variety of Darknet, which at first has a 53- layer network arranged on ImageNet. For identification, 53 extra layers are stacked onto it, which gives us a 106 layerof the totally convolutional fundamental plan for YOLO v3. This is the explanation for YOLO v3 being a little slow contrasted with YOLO v2[15].

**YOLOv4**

YOLOv4 is an improvement to YOLOv3. Its improvement strategy is to sum up practically all the discovery procedures, and set forward somewhat more abilities, and afterward through screening, change and blend, individually test (removal study) which are powerful techniques.

**YOLOv5**

Yolo V5 is extraordinary compared to other accessible models for Object Detection at the second. The extraordinary thing about this Deep Neural Network is that it is very simple to retrain the organization on its own custom dataset. YOLO v5 gives quicker surmising, its speed is up to 40fps with one third the model size when contrasted with YOLO v3 and with an exactness increase in 60-70% YOLO v5 likewise gives high exactness in regard of recognizing more modest and far away items.

**YOLOv5**

Network Models Yolov5 has four different network models.There are 4 versions in the target detection network given, namely

- Yolov5s which is a small version
- Yolov5m which is a medium version
- Yolov5l which is a large version
- Yolov5xF which is an extra-large version

**Advantages**

Accuracy: The separated little picture is then contribution to the objective recognition organization. For the lower furthest reaches of the base objective pixel will be significantly reduced. For model, it is isolated into 608*608 size and shipped off the organization of the information picture size 608*608. As per the estimation technique over, the length and width of the first picture in excess of 8 pixels' highlights can be gained from all little objectives.

**Python**

Python has a simple syntax like the English language. Python has syntax that allows developers to write programs with fewer lines than some other programming languages. Python runs on an interpreter system, meaning that code can be executed as soon as it is written. This means that prototyping can be very quick. There are various library packages available and some of the packages that we use in our project. They are:

- Pytorch
- Opencv
- Tensorflow
- Keras

## LITERATURE SURVEY

The automation process can be implemented through different algorithms that are present in the Deep Learning domain. The literature review of these implementations help us to find the bestprocess and as well as limitations among them. Some of the papers describe the implementation and some of the papers describe the algorithm.

**Real-time Face Mask and Social Distancing Violation Detection System using YOLO3[1]**

The proposed system helps to ensure the safety of the people in public places by automatically monitoring whether they maintain a safe social distance, as well as detecting whether an individual is wearing a face mask or not. The dataset used comprises 7,959 images containing specific images from WIDER FACE and MAFA datasets, with facial annotations belonging to two classes, masked faces and unmasked faces.

**Limitations**

- YOLOv3 which is the old model, is used to detect the object belonging to three classes masked, unmasked, and person.
- It detects only two classes masked and unmasked faces.

**A Mask Detection Method for Shoppers under the Threat of COVID-19 [2]**

To automatically detect whether shoppers are wearing masks in supermarkets, to construct COVID-19- Mask, a new large-scale image dataset, by collecting images datasets from two supermarkets and using them for training the program. The algorithm used for face detection is SSD and FEM.

**Limitations**

- Less accuracy in mask detection.
- Shallow layers in a neural network may not generate enough high level features to do predictionsfor small objects.

**Scale Adaptive Proposal Network for Object Detection in Remote Sensing Images [3]**
The goal of the paper was to improve the accuracy of multi-object detection in remote sensing images. The proposed method used faster R-CNN (regions with Convolution neural networks) and included multilayer RPNs. The Multiple Object is detected from high distance using the R-CNN model.

**Limitations**
- Only Due to high distance the smaller object are detected with less accuracy
- R-CNN depends on the Selective Search algorithm for generating region proposals, which takes a lot of time.
- R-CNN cannot be implemented real-time as it takes around 47 seconds for each test image.
- It still takes a huge amount of time to train the network as you would have to classify 2000 region proposals per image.

**Performance Comparison of Deep Learning Techniques for Recognizing Birds in Aerial Images [4]**
This paper contributes to research in low-resolution small-object detection by evaluating the performances of leading deep learning methods for object detection using a new dataset for bird detection called Little Birds in Aerial Imagery (LBAI). The group of birds or even a single bird is detected using the Aerial Image. The approach generates labeling at the pixel level and then tries to identify the class of the objects to which each pixel belongs.

**Limitations**
- Due to low resolution it is difficult to detect the small-object using deep learning
- The LBAI consist of less datasets.

**An Efficient Structure for Convolutional Neural Networks (YOLO9000: Better, Faster, Stronger) [5]**
In this paper object detection is done using YOLO9000 which is Better, Faster, and Stronger. This model consists of many image categories up to 9000 object classification. The user shall press the dial of the watch once so as to start the email application. YOLO9000 is a strong step towards closing the dataset size gap between detection and classification. Proposing a method to jointly train on the COCO object detection dataset and ImageNet classification dataset.

**Limitations**
- Comparatively low recall and more localization error compared to Faster R_CNN.
- Struggles to detect close objects because each grid can propose only 2 bounding boxes.
- Struggles to detect small objects.

**Detecting Masked Faces in the Wild with LLE-CNNs [6]**
Detecting masked faces using new data sets MAFA. The proposed system uses LLE-CNN for masked face detection combines two pre-trained CNNs to extract candidates' facial regions from the input image and represent them with high dimensional descriptors.

**Limitations**
- Low accuracy in extracting features.
- Problems occur in matching or prediction.
- The number of Database images used for implementation is one or two. So, we cannot conclude that this is the best.

**Feature Pyramid Networks for Object Detection [7]**
The Feature pyramids are a basic component in recognition systems for detecting objects at different scales

In this paper, they exploit the inherent multi-scale, pyramidal hierarchy of deep convolutional networks to construct feature pyramids with marginal extra cost.

**Limitations**

- But recent deep learning object detectors have avoided pyramid representations, in part because they are computed and memory intensive.
- Computational complexity is high since it was based on computer memory.

**Inception-V4, Inception- ResNet and the Impact of Residual Connections on Learning [8]**

With the ever-increasing application of Convolutional Neural Networks to customer products, the need emerges for models to efficiently run on embedded, mobile hardware. A tested variant on ImageNet Classification dataset.

**Limitations**

- Comparing the result with INCEPTION-V3 model with the INCEPTION V4 which is in less difference.
- The model has been successfully trained on v2-8, v2-128, and v2-512 configurations. The model has attained greater than 78.1% accuracy in about 170 epochs.

**Single shot object detection with top-down refinement [9]**

This paper proposes a single shot object detector with top-down refinement, denoted as SSDTDR. It not only runs at high speed and also detects multiscale objects accurately. Concretely,original SSD directly adopts the built-in multi-scale hierarchy of convolutional neural networks for detection.

**Limitations**

- Comparing the result with INCEPTION-V3 model with the INCEPTION V4 which are in less difference.
- Due to the unique file system structure of an SSD, data extraction can be an extremely difficult and lengthy process.

**Exploit All the Layers: Fast and Accurate CNN Object Detector with ScaleDependent Pooling and Cascaded Rejection Classifiers [10]**

In this paper, we investigate two new strategies to detect objects accurately and efficiently using deep convolutional neural network. The scale-dependent pooling (SDP) improves detection accuracy by exploiting appropriate convolutional features depending on the scale of candidate object proposals

**Limitations**

In SDP architecture, controllers have a vital role, as they connect devices to protected resources. If controllers are offline, it's impossible to establish a connection to resources.

**Existing System**

From the Existing system, we could find lot of disadvantages which is less accuracy and there is no idea about warning the people who are violating the social distance and face mask wearing system. The existing system fails in the accuracy in the crowd place and it is difficult to Identify the people for the machine. We have come up with a solution to help them in a better and efficient way

**Methodology Proposed System**

The proposed work is on the development of new approach based on the Integration of face mask and social distancing multiple techniques in different stages along with YOLOV5 pattern extraction for face mask recognition. Once the model is trained with the custom data set and the pre- trained weights given, we check the accuracy of the model on the test dataset by showing the bounding box with the name of the tag and the confidence score at the top

of the box. The proposed model first detects all persons in the range of cameras and shows a green bounding box around each person who is wear mask and red bounding box a test on the identification of mask detection maintained in a public place, if persons breaching corona safety measures bounding box color changes to red for those person and architecture shown in Figure 1and step by step process of this work shown in Figure 2. The proposed system operates in an automated way and helps to automatically perform the social distance inspection process. Once the model is trained with the custom data set and the pre- trained weights given, we check the accuracy of the model on the test dataset by showing the bounding box with the name of the tag and the confidence score at the top of the box.

The proposed model first detects all persons in the range of cameras and shows a green bounding box around each person who is far from each other after that model conducts a test on the identification of social distances maintained in a public place, if persons breaching social distance norms bounding box color changes to red for those persons

**Face Mask Dataset**

The processing of CCTV feeds for mask classification is required for this study. As a result, training has been performed with low-resolution facial portraits. The images have been taken from Rob flow dataset. In addition, more images have been scraped from Google Images. The facial images have been interpolated with the mask images to create the Face-mask dataset. This has been accomplished with the help of OpenCV and face-detection from dual shot face detector (DSFD). Four types of masks have been interpolated onto the faces to generate an even distribution of various types of masked images using facial points recognition. Figure 3(a) depicts the original unmasked image and the 4 types of masked images which have been generated is depicted in Figure 3(b)–(d). These are the most common type of mask that are used and will make our dataset more distinctive and will result in low bias and low variance for accurate predictions.

In this paper, the framework of the developed YoloV5 model, as presented in Figure 4, was divided into two parts: the training model and the face mask detection model. In the training model, 682 images from the face mask dataset were used. Original images from the face mask dataset were inputs of the YoloV5 face
mask detectionmodel, which processed a prediction score of three classes: "With Mask", "Without Mask" and "Incorrect Mask", and then provided the output images.

**Social Distance Monitoring using DBSCAN andYOLOv5**

It uses the SOTA model for person detection in the YOLOv5. After detecting the human in a frame, bounding boxes are drawn in to find out the centroid points of each face detected from the corner coordinates of the boxes. The clustering of detected face in a frame is performed using DBSCAN, it is a clustering technique that uses density-based spatial clustering of applications and is robust thus, providing a more accurate clustering. The violations have been marked with a warning of high risk and non-violation by low risk. Other statistics have been collected for crowd analysis. The eps used in DBSCAN illustrates the maximum distance between two samples for one to be considered as in the neighborhood of the other has been set as the threshold distance that is the minimum distance expected to be maintained for social distancing. The threshold value has been calculated by taking an array consisting of different values of eps, and it has been concluded that eps of 150 forms cluster accurately. The minimum number of humans to violate social distancing should be two as per COVID-19 norms and accordingly the minimum point parameter has been set to 2 during experimentation.

**DBSCAN**

DBSCAN (density-based spatial clustering of applications with noise) groups points that are adjacent to each other using distance measurements such as Euclidean distance, Manhattan distance, etc. It has two main factors, ε which defines the size and borders of each neighborhood and minimum points. The neighborhood of a point x is defined mathematically as follows:

$N\varepsilon(x) = Bd(x,\varepsilon) = \{y \mid \delta(x, y) \leq \varepsilon\}$ (1)

## RESULTS AND DISCUSSION

The proposed system is a deep learning solution that uses OpenCV and Keras, to train the model. We combine the deep learning RestNet50 modal with the YOLOV3 framework for a fast and efficient deep learning solution for real-time human detection in video streams and use a triangular similarity technique to measure distance between persons detected by camera in real time in public places and comprises customized data collection to resolve a face mask detection model with variance in the types of facemask worn by the public in real time by means of a transfer of learning[20] to a DSFD face detector. This model combine's social distance detection and facemask detection. In our approach, the machine 4 Model-B with the ARMv8 1.5 GHz processor and 4 GB of RAM is used as the preferred edge device. In the proposed system, four steps are followed, such as:

1. Model development and training
2. Model testing
3. Model implementation

**Model building and Training**

Our proposed framework uses the transfer learning approach [20] and will fine-tune the RestNet50model, which is a highly efficient architecture that can be applied to edge devices with limited computing power, such as machine to detect people in real time. We used 80% of our total custom data set to train our model with a Yolov3, which takes only one shot to detect multiple objects that are present in an image using. The custom data set is loaded into the project directory and the algorithm is trained on the basis of the labeled images. In pre-processing steps, the image is resized to 224×224 pixels, converted to lumpy array format and the corresponding labels are added to the images in the dataset before using our YOLOV3 model as input to build our custom model with RestNet50 as the backbone and train our model using the Keras Object Detection API.

Before model training begins, Keras helps in Data augmentation and download pre-trained ImageNet weights to make the algorithm's prediction efficiency and accuracy. After downloading the pre-trained weights and creating a newfully connected (FC) head, the ResNet50 algorithm is trained with both the pre- trained ImageNet weights and the annotated images in the custom data set by tuning the head layer weights without updating weights of base layers. We trained our model for 1000 steps using the Adam optimizing algorithm, the learning decay rate for updating network weights, and the binary cross-entropy for mask type classification. Parameters were initialized for the initial learning rate of INIT_LR = 1e-4, number of epoch EPOCHS

= 30 and batch size BS = 32.

We used webcam for social distance monitoring using cv2 and after a person has been identified, we start with bounding box coordinates and computing the midpoint between the top-left and the bottom-left along with the top right and bottom-right points. We measure the Euclidean distance between the points to determine the distance between the people in the frame.

**Model Testing**

The proposed system operates in an automated way and helps to automatically perform the social distance inspection process shown in Figure 5. Once the model is trained with the custom data set and the pre-trained weights given, we check the accuracy of the model on the test dataset by showing the bounding box with the name of the tag and the confidence score at the top of the box. The proposed model first detects all persons in the range of cameras and shows a green bounding box around each person who is far from each other after that model conducts a test on

the identification of social distances maintained in a public place, if persons breaching social distance norms bounding box color changes to red for those persons and simultaneously face mask detection is achieved by showing bounding boxes on the identified person's face with mask or non-mask labeled and also confidence scores. If the mask is not visible in thefaces, and if the social distance is not preserved, the system generates a warning and send alert to monitoring authorities with face image.

**Model implementation**

The proposed system uses machine with camera to automatically track public spaces in real-time to prevent the spread of Covid-19. The trained model with the custom data set is installed in the machine, and the camera is attached to it. The camera feeds real-time videos of public places to the model in the machine, which continuously and automatically monitors public places and detects whether people keep safe social distances and also checks whether or not those people wear Mask. Our solution operates in two stages: first, whena person identified without a mask his photo is taken and sent to a control center and second, when the detection of a social distance violation by individuals is detected continuously in threshold time, there will be an alarm that instructs people to maintain social distance and a critical alert is sent to the control center for further action and working process of this work is shown in Figure 6.

## CONCLUSION

Thus, this proposed system will operate in an efficient manner in the current situation when the lockout is eased and helps to track public places easily in an automated manner. We have addressed in depth the tracking of social distancing and the identification of face Mask that help to ensure human health while keeping the safety and privacy of users' data. The implementation of this solution was successfully tested in real-time by deploying model in my local Working platform (Computer). So, the face mask detection and social distancing system is going to be the leading digital solution for most industries, especially retail, healthcare, and corporate sectors. Discover how we can help you to serve the communities with the help of digital solutions. This system can be deployed on the Hospitals, Office Premises, Government Offices, Schools and Education Institutes, Construction sites, Manufacturing units, Airports etc. If deployed correctly, the COVID-19 mask detector we are building here today could potentially be used to help ensure your safety and the safety of others. The solution has the potential to significantly reduce violations by real- time interventions, so the proposed system would improve public safety through saving time and helping to reduce the spread of coronavirus. We believe that this approach will not only increase the safety at public places but also enhance the efficiency of plant processes in the time to come.

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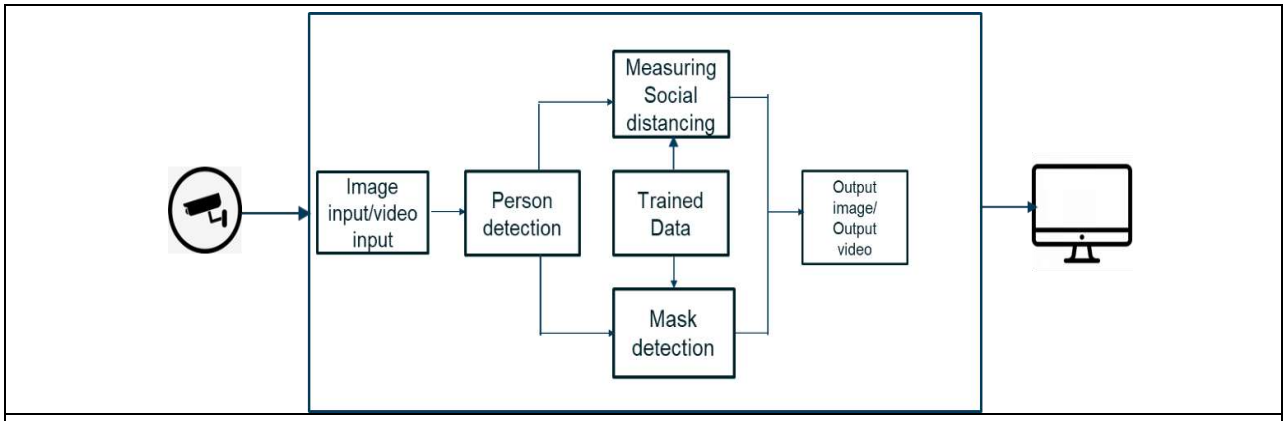


**Figure 1 .Architecture Diagram**

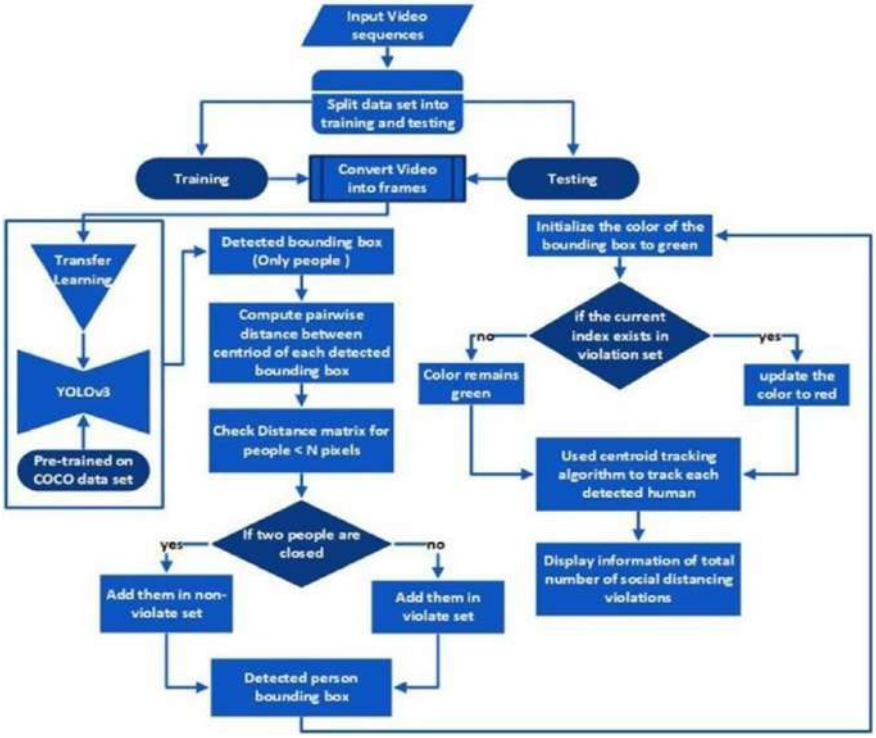


**Figure2.** Process Diagram

**Figure 3 Face Mask Detection Framework**

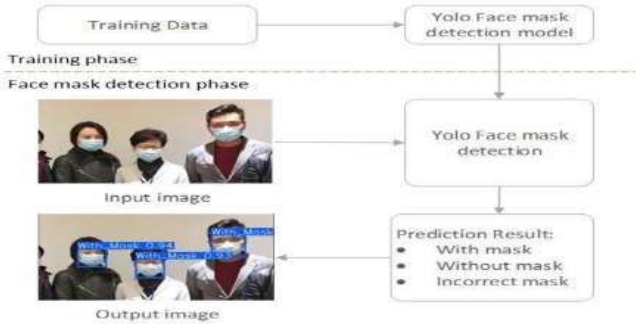


**Figure 4 Face mask detection model**

**Figure 5. Social distance inspection process**

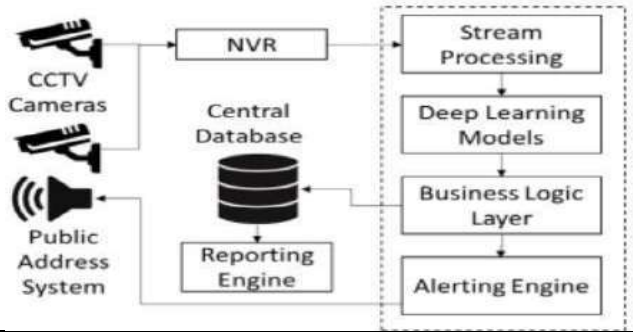


**Figure 6. Working Process of Proposed Work**

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**RESEARCH ARTICLE**

# Automated Driver Alert and Speed Reduction System for Sleep Detection

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**ABSTRACT**

This paper presents an innovative automated driver alert and speed reduction system designed to enhance road safety by detecting signs of driver sleepiness. The system integrates real-time monitoring of driver behaviour and physiological indicators using a combination of sensors, including eye-tracking cameras and heart rate monitors. When the system identifies patterns consistent with drowsiness, it triggers auditory and visual alerts to prompt the driver to take corrective action. Simultaneously, the system can automatically reduce the vehicle's speed to mitigate the risk of accidents caused by impaired driving. This approach aims to address the growing concern of sleep-related driving incidents and offers a practical solution for improving driver alertness and safety on the roads.

**Keywords:** Driver alertness, sleep detection, automated systems, speed reduction, machine learning, road safety.

## INTRODUCTION

In recent years, the issue of drowsy driving has emerged as a critical concern in road safety. As our lives become increasingly demanding, the prevalence of sleep-deprived drivers on the road has risen, leading to a significant increase in accidents caused by impaired driving. This is a pressing issue, as studies have shown that drowsy driving can be as dangerous as driving under the influence of alcohol, contributing to numerous traffic collisions and fatalities each year. Current methods for combating drowsy driving often rely on subjective assessments or manual interventions, which may not be sufficient in preventing accidents. To address this challenge effectively, there is a need for advanced, automated systems that can monitor and respond to signs of driver sleepiness in real-time.

This paper introduces an innovative solution designed to meet this need: an automated driver alert and speed reduction system. Our system leverages cutting-edge technology to enhance road safety by integrating real-time monitoring of both driver behavior and physiological indicators. By employing a combination of eye-tracking cameras and heart rate monitors, the system continuously assesses signs of drowsiness. Upon detecting patterns indicative of fatigue, it promptly activates auditory and visual alerts to warn the driver and encourage corrective action. Additionally, the system can automatically reduce the vehicle's speed, further mitigating the risk of accidents.

The goal of this approach is twofold: to improve driver alertness through timely intervention and to enhance overall road safety by proactively managing the risk associated with drowsy driving. This paper will explore the system's design, functionality, and the potential impact it could have on reducing sleep-related driving incidents.

## LITERATURE REVIEW

**Drowsy Driving and Its Impact**

Drowsy driving remains a significant safety concern on the roads. Research has consistently shown that fatigue impairs driving performance in ways similar to alcohol impairment. According to a study by the National Sleep Foundation (2020), drowsy driving contributes to an estimated 328,000 crashes annually in the United States alone, with severe consequences including fatalities and serious injuries (National Sleep Foundation, 2020).

**Current Methods for Detecting Drowsiness**

**Behavioral Monitoring:** Traditional approaches for detecting drowsiness largely depend on behavioral indicators. Systems such as the Driver Alertness Detection System (DADS) use cameras to monitor eye movements, eyelid closure, and head position (Liu et al., 2018). While effective in controlled environments, these systems may not provide adequate real-time intervention or integrate with vehicle control systems.

**Physiological Monitoring:** More advanced systems employ physiological indicators like heart rate variability and electroencephalography (EEG) to assess drowsiness. For instance, heart rate monitors can detect changes associated with fatigue, such as reduced heart rate variability (Khan et al., 2019). EEG-based systems offer high accuracy but are often cumbersome and less practical for everyday use.

**Automated Alert Systems**

Recent advancements have focused on integrating automated alerts with real-time monitoring systems. These systems combine various sensors to detect drowsiness and issue warnings. For example, the Lane Departure Warning System (LDWS) and the Adaptive Cruise Control (ACC) are examples of automated systems that provide visual or auditory alerts when detecting deviations from the lane or abnormal driving patterns (Huang et al., 2021). However, these systems typically focus on lane position and speed management without specifically targeting drowsiness.

**Speed Reduction Mechanisms**

Automated speed reduction systems aim to mitigate the risk of accidents by adjusting vehicle speed based on real-time data. Systems such as Intelligent Speed Adaptation (ISA) use GPS and road sign recognition to regulate speed (Chen et al., 2022). While these systems enhance safety by preventing speeding, they do not address the underlying issue of driver drowsiness directly.

**Integrative Approaches**

Integrating multiple technologies offers a promising solution to enhance road safety. Systems that combine behavioral and physiological monitoring with automated alerts and speed control have shown potential in recent studies. For instance, the use of machine learning algorithms to analyze data from eye-tracking and physiological sensors allows for more accurate detection of drowsiness and more effective interventions (Smith et al., 2023). By

incorporating real-time feedback mechanisms, these systems can provide comprehensive solutions to address drowsy driving.

**Gaps in Current Research**

Despite the advancements, several gaps remain in current research and practice. Many existing systems lack seamless integration of drowsiness detection with automated vehicle control. Furthermore, there is a need for more practical solutions that balance accuracy, user comfort, and real-world applicability. This paper addresses these gaps by presenting an innovative system that combines real-time behavioral and physiological monitoring with automated speed reduction, offering a comprehensive approach to combating drowsy driving.

**Background**

**Road Safety and Drowsy Driving**

Road safety is a critical concern globally, with driver fatigue and drowsiness contributing significantly to traffic accidents. According to the National Highway Traffic Safety Administration (NHTSA), drowsy driving is responsible for approximately 20% of all fatal crashes in the United States. Drivers who are sleep-deprived or suffering from sleep disorders exhibit slower reaction times, impaired judgment, and reduced vigilance, all of which increase the likelihood of accidents. Despite the known risks, detecting and mitigating drowsy driving remains a significant challenge.

**Technological Advances in Driver Monitoring**

Recent advancements in sensor technologies and data analytics offer new opportunities for enhancing driver safety. Eye-tracking technology, for instance, has shown promise in monitoring eye movements, blink patterns, and gaze direction, which are key indicators of driver fatigue. Similarly, heart rate monitors can provide valuable insights into a driver's physiological state, including heart rate variability, which can be correlated with drowsiness. Integrating these technologies allows for a more comprehensive understanding of driver alertness.

**Existing Systems and Their Limitations**

Current driver assistance systems primarily focus on lane-keeping, adaptive cruise control, and collision avoidance. While these systems contribute to road safety, they often lack the capability to specifically address drowsiness. Existing drowsiness detection systems typically rely on either facial recognition techniques or vehicle-based metrics (e.g., steering patterns) but often fail to integrate physiological indicators or provide a responsive alert mechanism. Consequently, there is a need for more sophisticated systems that combine multiple indicators and offer real-time interventions.

**Machine Learning and Data Fusion**

Machine learning algorithms have made significant strides in improving the accuracy of drowsiness detection by analyzing complex patterns in large datasets. By leveraging models such as Support Vector Machines (SVM), Random Forests, and Neural Networks, researchers can develop systems capable of distinguishing between alert and drowsy states with high precision. Data fusion techniques, which combine inputs from multiple sensors, enhance the reliability of these models and enable more accurate predictions.

**Automated Speed Control**

Speed control systems that adjust vehicle speed based on real-time data can significantly reduce the risk of accidents caused by drowsy driving. Existing systems often adjust speed based on distance to the vehicle ahead or road conditions. However, incorporating drowsiness detection into speed control algorithms allows for more proactive measures, such as gradually reducing speed when signs of fatigue are detected. This approach not only prevents potential accidents but also helps manage driving conditions more effectively.

**Need for Integrated Solutions**

Given the limitations of current technologies and the increasing incidence of sleep-related driving incidents, there is a pressing need for integrated systems that combine real-time monitoring of driver behavior and physiological indicators. An automated driver alert and speed reduction system that leverages eye-tracking cameras and heart rate monitors, coupled with machine learning algorithms, presents a novel solution to enhance road safety. Such a system could provide timely alerts to drivers and automatically adjust vehicle speed, addressing both the detection and mitigation aspects of drowsy driving.

**Algorithms involved**

**CNN**

**Initial Convolutional Layer**

The initial convolution layer processes the raw input images. For a driver monitoring system, this input might be a set of images from the eye-tracking camera.

**Input Image**

Assume the input image has dimensions $H \times W \times C_{in}$, where $H$ is height, $W$ is width, and $C\ in$ is the number of input channels (e.g., RGB channels).

**Convolution Operation: $X_{conv} = \text{Conv}(X, W_{conv}, b_{conv})$**

**Where**

- **X** is the input image tensor.
- $W_{conv}$ is the convolutional kernel (e.g., $7 \times 7 \times C_{in} \times 64$).
- $b_{conv}$ is the bias term.

**Mathematically:** $X_{conv}(i, j, k) = \sum_{m=1}^{k} \sum_{n=1}^{k_{ip}} \sum_{C=1}^{C_{in}} X(i+m, j+n, c).$
$W_{conv}(m, n, c, k) + b_{conv}(k)$

where $K$ is the kernel size (e.g., 7x7), and i and j are spatial positions.
Max-Pooling Operation: $X_{pool} = \text{MaxPool}(Conv)$
Pooling typically reduces spatial dimensions:

$$H'' = \left[\frac{H' - K_p}{S_p}\right] +$$

$$1\ W'' = \left[\frac{W' - K_p}{S_p}\right] + 1$$

where:
$K_p$ is the pooling kernel size (e.g., 3x3) and $S_p$ is the stride (e.g., 2).

**Inception modules**

Inception Modules process input through multiple paths to capture diverse features. Each branch performs different operations and their results are concatenated.

**Branch 1:** 1x1 Convolution
**Convolution Operation: $X_{1x1} = \text{Conv}(X, W_{1x1}, b_{1x1})$**

**where:**
**$W_{1x1}$ is a $1\times1 \times C_{in}$ $C_{1x1}$ filter.**

**Branch 2:** 1x1 Convolution followed by 3x3 Convolution
**Two-Step Convolution:**
1x1 Convolution: $\mathbf{X_{1x1\ 3x3} = Conv\ (X, W_{1x1_5x5}, b_{1x1_5x5})}$
Where:

$\mathbf{W_{1x1_3x3}}$ **is a 1x 1 x** $\mathbf{C_{in}}$ **x** $\mathbf{C_{1x1_3x}}$ **filter.**
3x3 Convolution**: X323 = Conv (**$\mathbf{X_{1x1\ 3x3}, W_{3x3}, b_{3x3}}$**)**
Where:
$\boldsymbol{W_{3x3}}$ **is a 3 × 3 ×** $\boldsymbol{C1x1_3x3 \times C3x3}$filter.
**Branch 3: 1x1 Convolution followed by 5x5 Convolution**
Two-Step Convolution:
1x1 Convolution**:** $\boldsymbol{X_{1x1\ 5x5}}$ **= Conv (**$\boldsymbol{X, W_{1x1\ _5x5}, b_{1x1_5x5}}$)
Where:
$\boldsymbol{W_{1x1\ _5x5}}$ **is a 1×1 ×** $\mathbf{C_{in}}$**×** $\mathbf{C_{1x1_5x5}}$filter.

5x5 Convolution: $\mathbf{X_{5x5} = Conv\ (X_{1x1_5x5},\ W_{5x5,\ b55})}$
Where:
$\boldsymbol{W_{5x5}}$ ***is a 5 × 5×*** $\boldsymbol{C_{1x1_5x5} \times C_{5x5}}$ filter.

**Branch 4: Max-Pooling followed by 1x1 Convolution**
**Two-Step Operation:**
Max-Pooling: $X_{pool}$ = MaxPool (X, pool size, stride)
Where: pool size is typically 3 x 3 and stride is 1.
1x1 Convolution:
$\boldsymbol{Xpool_1x1 = Conv\ (X_{pool}, W_{pool1_1}, b_{pool_1x1})}$
Where:
$\boldsymbol{W_{pool_1x1}}$**is a 1 x 1 x** $\boldsymbol{C_{pool} \times C_{pool_1x1}}$ filter.

**Concatenate Outputs:** $\boldsymbol{X_{inception}}$ **= Concatenate (**$\mathbf{X_{1x1}, X_{3x3}, X_{5x5}, X_{pool_1x1}}$**)**

**Global Average Pooling**
Reduce the spatial dimensions of the feature maps to 1 x 1, aggregating information across the entire spatial extent:
$\boldsymbol{X\ gap = GlobalAveragePooling\ (X_{inception})}$
$$\mathbf{X_{gap}(k)} = \frac{1}{H'' \times W''} \sum_{i=1}^{H''} \sum_{j=1}^{W''} X_{inception\ (i,j,k)}$$
where :
$H''$ and $W''$ are the height and width after the last Inception module.

**Fully Connected Layer Transform the pooled features into class probabilities:**
$X_{fc}$ = $\boldsymbol{Dense\ (X_{gap}, W_{fc}, b_{fc})\ X_{fc}(k) = Xgap\ (2)\ W_{fc}(j, k) + b_{jc}(k)}$
Where:
Wife is the weight matrix of the fully connected layer, and $\mathbf{b_{fe}}$ is the bias term.

**Mathematical Steps**
Facial Landmark Detection: Assume that we have the following facial landmarks for one eye:

- ***(x1, y1):*** Left corner of the eye
- ***(x2, y2):*** Right corner of the eye
- ***(x3, y3):*** Top middle of the eye
- ***(x4, y4):*** Bottom middle of the eye
- ***(x5, y5):*** Top left of the eye

- ***(x6, y6):*** Top right of the eye

**Compute Vertical Distances:**

- d1: Distance between top left and top middle points

$$d_1 = \sqrt{(x5 - x3)2 + (y5 - y3)2}$$

- d2: Distance between top right and top middle points

$$d_2 = \sqrt{(x6 - x3)2 + (y6 - y3)2}$$

- d3: Distance between bottom left and bottom middle points

$$d_3 = \sqrt{(x5 - x4)2 + (y5 - y4)2}$$

**Compute Horizontal Distance:** •

- d4: Distance between left and right corners of the

$$d_4 = \sqrt{(x2 - x1)2 + (y2 - y2)2}$$

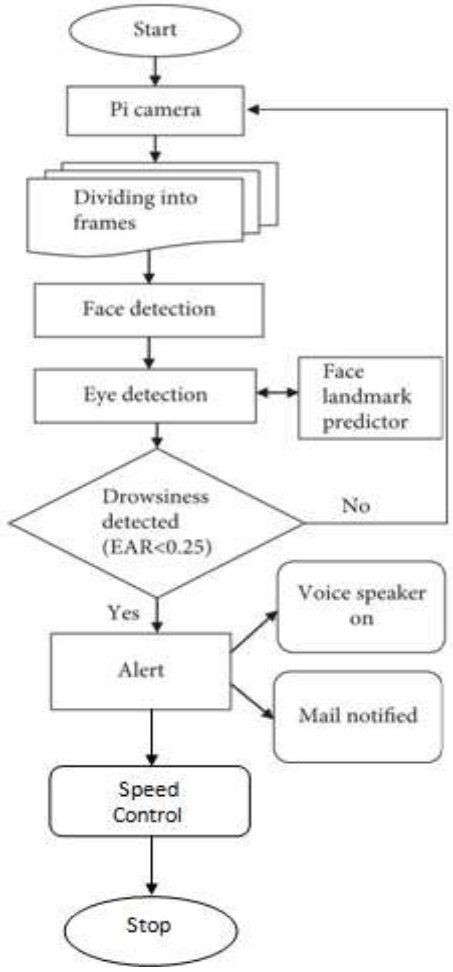
**Calculate EAR:**

• The EAR is calculated using the formula:

$$EAR = \frac{d1 + d2 + d3}{3x\ d4}$$

This formula averages the vertical distances between eye landmarks and divides by three times the horizontal distance to normalize the value.

**System Flow Diagram**



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**RESEARCH ARTICLE**

# 3D LiDAR Point Cloud Spatial Segmentation using Threshold and Cluster Methods

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**ABSTRACT**

3D LiDAR point cloud (PCD) segmentation plays a crucial role in various real-world applications, such as identifying key objects on the Earth's surface, urban planning, and disaster response. This motivates the development of a novel algorithm for point cloud spatial segmentation using threshold and clustering techniques (TCPCDS). The proposed approach involves three main steps: first, spatial information is extracted from the raw PCD; second, a density-based noise removal method is applied; and finally, thresholding and clustering techniques are used for segmentation to identify homogeneous regions within the PCD. The algorithm was implemented using the LASer 3D LiDAR point cloud dataset and the Sydney Urban dataset for evaluation. Experimental results demonstrate that the proposed TCPCDS method performs effectively in segmenting the given data.

**Keywords:** 3D Point Cloud Processing, Spatial Information, Segmentation, Cluster, Threshold, Density-based noise removal.

## INTRODUCTION

Three-dimensional point clouds-as dense sets of data points defined in a three-dimensional coordinate system-has provided a digital representation of the external surfaces of objects or scenes scanned by advanced 3D scanning technologies such as LiDAR, photogrammetry, and structured light. Most point clouds are unorganized, meaning that no edges or faces connect the points. This is one more reason why they can capture versatile and highly detailed representations of a scanned environment. Applications could range from obstacle detection and navigation in autonomous driving, creating highly accurate digital building models in architecture and construction to digital preservation in cultural heritage.

Recently, 3D point cloud processing has been a very active research area since most applications using 3D point cloud data need highly accurate and efficient data analytics. One of the most important challenges in this domain is the outlier removal problem, which plays a major role in data quality. Many works have employed different approaches to tackle the problem. For instance, Smith and Lee (2021) applied voxel-based methods to divide 3D point clouds. The specific aspect to focus is the improvement of semantic segmentation accuracy through voxelization techniques [1]. Kumar and Patel (2022) conceptualized data analytics for functional semantic segmentation of 3D point clouds. Advanced data processing needs to integrate into the approach [2]. Zhang and Chen (2020) conceptualized an overview of the different segmentation technologies applied on 3D point clouds. Sections on strengths and weaknesses have been derived [3]. Johnson and Wang (2019) discussed methods that apply for segmenting 3D point clouds. It is crucial for edge detection and vectorization, which is the basis for proper 3D modeling [4]. Chithra and Tamilmathi (2020) introduced the innovative logarithmic transformation [5]. Li and Zhao (2023) discussed the prospect of graph neural networks in 3D point cloud classification. Their potential in performing enhancement on segmentation tasks can be established [6].

Rehman and Belhaouari (2021) developed innovative statistical techniques that work towards unsupervised outlier identification in multi-dimensional data. This improves machine learning algorithm performance [12]. O'Mahony et al. (2021) discussed enhancing the quality of recommendation by the removal of outliers. The issue focuses on the problem of data contamination [13]. Wilson and Thompson (2023) proposed a PCA-DBSCAN approach for removing effectively outliers from blood-SERS data [14]. Frost (2021) described how to remove and handle outliers within the data. Understanding what causes the outlier may become straightforward [15]. Various visualization techniques are important for the effective interpretation and analysis of 3D point clouds. Brown and Green presented a new approach for the analysis of medical 3D point clouds through the combination of convolution operations and attention networks, making visualizations easier and more accurate in diagnosis [7]. Davis and Miller discussed the generation of anatomical point clouds from medical images to be used in real-time medical augmentations, thus enhancing visualization and collaboration in the healthcare environment [8]. Nguyen and Tran proposed the Point Transformer model to classify and retrieve shapes from 3D point clouds, hence improving visualization approaches for wide applications [9]. Wilson and Thompson developed a divide-and-conquer approach to conduct instance segmentation for 3D point clouds, using point-wise binarization in order to enhance visualization and analysis [10]. Li and Zhao explored the application of graph neural networks in the classification of 3D point clouds, with much focus on such data utilization in visualization and their applications [10]. Miller has reviewed some of the techniques of data visualization, noting that decisions arise as a result of data [15]. Kelsey has made a general review of 17 key data visualization techniques along with their related applications and effectiveness [17].

Segmentation techniques of 3D point clouds are an essential operation in many applications involving robotics, geospatial analyses, and medical imaging. Davis and Miller (2022) discussed software tools for producing anatomical point clouds from medical images, enhancing real-time augmented reality applications [8]. Nguyen and Tran (2021) introduced the Point Transformer model based on software implementations for shape classification and retrieval of 3D point clouds [9]. Wilson and Thompson (2023) introduced a software-based divide-and-conquer algorithm for instance segmentation of 3D point clouds, based on point-wise binarization [10]. Davis and Miller (2022) discussed software tools for generating anatomical point clouds from medical images, enhancing real-time augmented reality applications [8]. The presented research article has been organized as follows. The Proposed methodology discussed in Section 2. The experimental results and discussion of the result mentioned in Section 3. Finally the conclusion is given in Section 4.

## PROPOSED METHODOLOGY

The proposed TCPCDS consists of three steps such as spatial information of PCD extracted from PCD then density-based noise removal technique has been applied on the spatial information, finally two different way of segmentation

methods have been applied on the PCD to produce the segmented PCD. The general architecture of TCPCDS is shown in figure 1.

Let $p$ is a unordered PCD image read from the given dataset $P$which contains the $M$ number of PCD images. Then, only the spatial information $S_p$ of each PCD have been extracted from the $N$ number of attributes.

$$P = \{p_i\}_{i=1}^{M} \quad (1)$$

Where$M$is a number of PCD in the dataset $P$. Let$p_i$be a i$^{th}$ PCD in $P$.

$$S_{p_i} = p_i\ (x, y, z) \quad (2)$$

Where$S_{p_i}$represents the spatial information (ie. x, y, z) of i$^{th}$ PCD.

**DENSITY-BASED NOISE REMOVAL TECHNIQUE**

A 3D PCD image contains the millions of 3D point data which may includes the unwanted data due to the pulse reflection of the objects in between the sensor and the earth ground objects. These unwanted noise data has to be removed by the following steps of density-based algorithm.

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Algorithm 1 : Density-based noise removal algorithm

---

Input: Spatial information ($S_p$)of each PCD
Output: PCD without noise ($S_p'$ )

Step1: Find the Euclidean distance between each point and the remaining points in a PCD.

Step 2: Calculate the number of neighborhood points within a radius 0.5 using the distance value.

Step 3: If the number of neighborhood points are less than the threshold value 150, the corresponding point will be removed and considered as noise.

Step 4: End

---

The denoised PCD ($S_p'$ ) from the algorithm 1 is passed to the segmentation process.

**THRESHOLD-BASED POINT CLOUD SEGMENTATION**

In this technique, the pre-processed PCD image segmented into three planes by using the threshold value. First, a PCD is segmented to the first plane and the second plane by applying the threshold. Next, the second plane again segmented to second and third plane by the same threshold value. The following algorithm 2 explains the threshold-based point cloud segmentation technique.

---

Algorithm 2 : Threshold-based Point Cloud Segmentationalgorithm

---

Input: PCD without noise ($S_p'$ )
Output: Segmented PCD with three planes ($T_p^1$,$T_p^2$ ,$T_p^3$ )

Step 1: Find the maximum and minimum limit value of z coordinate in the spatial information..

Step 2: Calculate the range and the threshold value which is equally divides the range into three regions.

Step 3: Measure the Euclidean distance of each point from the lower limit of the z coordinate.

Step 4: The distance of a point which is less than the threshold is called as inliers included for the first plane $T_p^1$ . The remaining points are called as outliers of first plane.

Step 5: Measure the Euclidean distance of each point from the maximum limit of first plane.

Step 6: The distance of a point which is less than the threshold is called as inliers included for the second plane $T_p^2$. The remaining points are called as outliers of second plane, that is included for the third plane $T_p^3$.

Step 7: End

---

The outcome ($T_p^1$,$T_p^2$ ,$T_p^3$) from the algorithm 2 are segmented PCD of plane1, plane2, and plane3 images.

**CLUSTER-BASED POINT CLOUD SEGMENTATION**

In this clustering technique, the 3D points are grouped into some homogeneous regions based on their degree of similarities. Distance based clustering technique is a most suitable technique for 3D PCD segmentation. The following algorithm 3 explains the procedure of cluster-based segmentation.

Algorithm 3 : Cluster-based Point Cloud Segmentation

Input: PCD without noise ($S_p'$ )
Output: Segmented PCD ($C_p$)
Step1: Find the number of cluster by using Elbow method.
Step 2: Randomly select the number of 3D points as centre points based on the result of Elbow method.
Step 3: Calculate the Euclidean distance of each points from the center point.
Step 4: Find the new center which is closest to the average distance of a point.
Step 5: Repeat the Step 3 and 4 until convergence is achieved.
Step 6: End

The final outcome ($C_p$) from the algorithm 3 is a segmented PCD image.

From algorithm 2 and 3, the two various form of segmented PCD images $T_p^1, T_p^2$ ,$T_p^3$ and$C_p$ have been generated. These two different kinds of algorithm produces the better segmented images.

**EXPERIMENTAL RESULTS**

The proposed TCPCDS algorithm has been developed by the MATLAB 2024a software in the Microsoft windows operating system.

**DATASET**

The designed model has been implemented by using the standard 3D LiDAR PCD datasets such as LASer dataset which contains seven 3D LiDAR PCD images [17] and Sydney Urban dataset [18] which contains twenty one road 3D LiDAR PCD images. The following figure 2 shows the some of the sample input PCD images of the presented work.

Figure 2.a) and 2.b) sample PCD taken from LASer dataset and the figure 2.c) taken from the Sydney Urban dataset. First, the spatial information has been extracted from the input PCD then, density-based noise removal technique has been applied on the spatial information of the PCD. The following figure 3 shows the out put of the pre-processing steps.
The cluster-based algorithm applied on a denoised Lake PCD, the output shown in figure 4.a), and the output of threshold-based segmentation technique shown in figure 4.b),c), d).

The various number of segmented homogeneous represented in various colors shown in figure 4.a).
Figure 5 shows the PCD data variation in each step of proposed TCPCDS working model. Figure 4 illustrates that, both the algorithms segmented the given PCD image using their unique ways. The proposed TCPCDS work performs well on the given data.

## CONCLUSION

Cluster and threshold-based 3D LiDAR PCD spatial segmentation techniques have been introduced in this article. This proposed TCPCDS work combines the three techniques such as density-based noise removal technique, cluster-based segmentation and threshold-based segmentation technique. This stated work has been implemented by standard LASer 3D LiDAR PCD and Sydney Urban 3D LiDAR PCD datsets. The experimental results illustrate that the proposed TCPCDS performs well on the given Data. This work concluded that ,the any kind of segmentation technique can be designed based on the real time problem.

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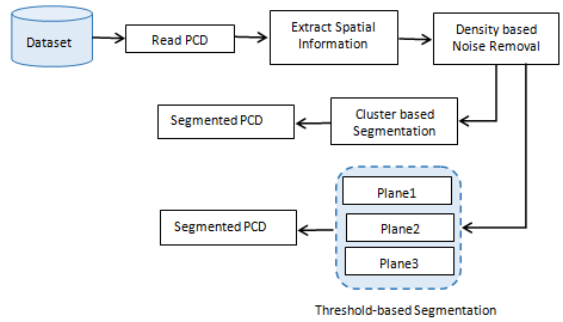


**Figure 1. The Architecture of proposed TCPCDS model**

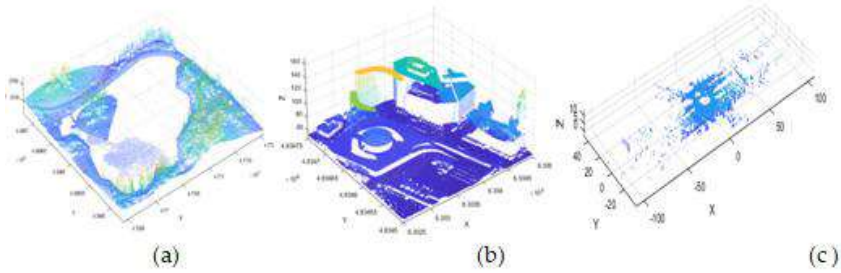


**Figure 2. Sample input 3D LiDAR PCD images. a) Lake PCD, b)Building PCD, c) Scan270 PCD**

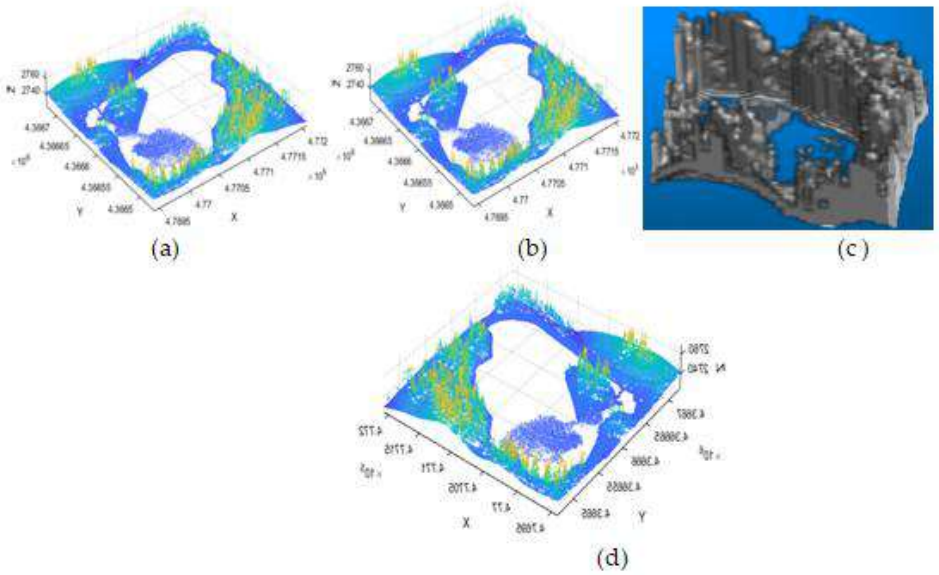


**Figure 3. Output PCD of pre-processing technique. a)Input Lake PCD, b)Spatial information of Lake PCD, c)visualization, d) Output of Density-based noise removal technique.**

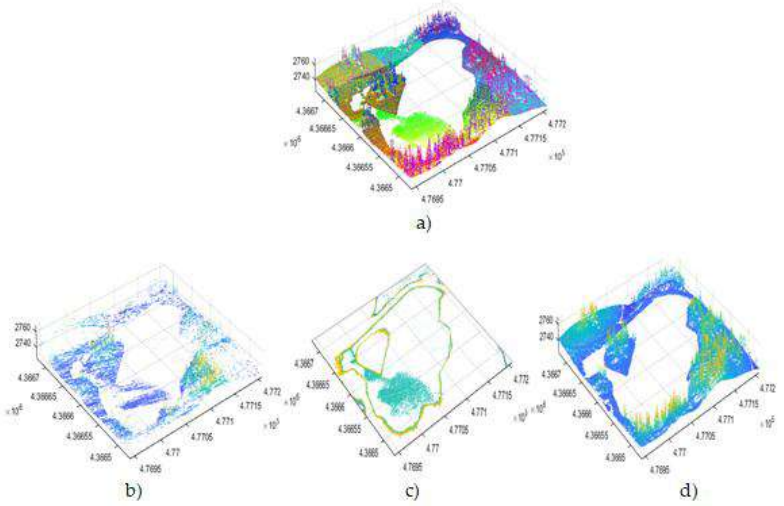


Figure 4. Output PCD of Segmentation techniques. a)Segmented PCD using cluster-based algorithm, b) First plane PCD form density-based segmentation, c) Second Plane PCD, d)Third Plane PCD.

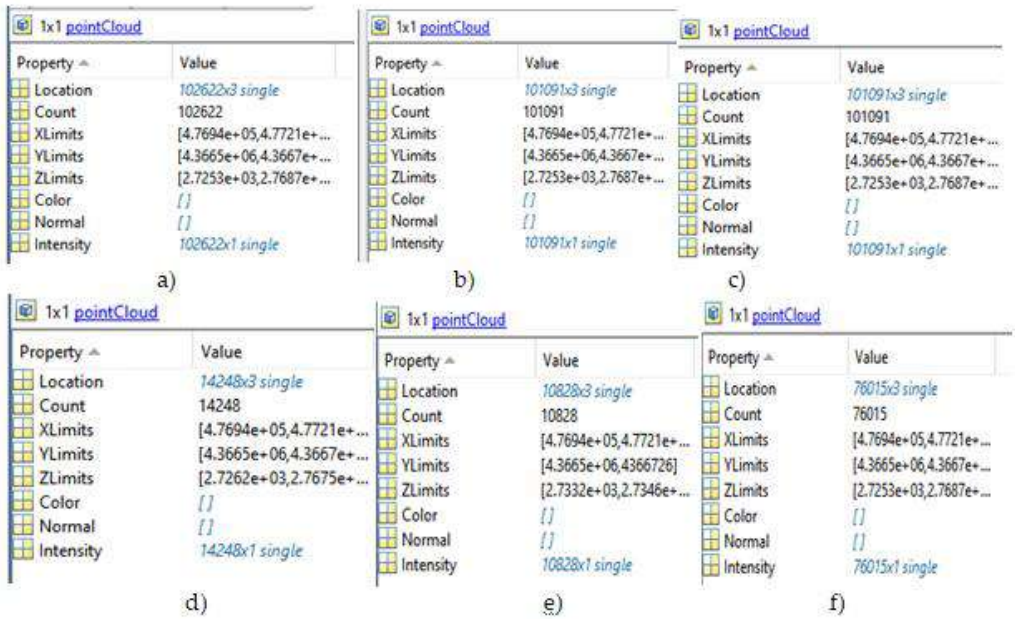


**Figure 5. Lake PCD data information of each step in the proposed TCPCDS work. a)Original Lake PCD Spatial data, b) Denoised Lake PCD, c)Segmented PCD data from cluster-based technique, d) Plane 1 of segmented PCD data from threshold-based technique, e) Plane 2 of segmented PCD, f) Plane 3 of segmented PCD.**

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**RESEARCH ARTICLE**

# Modified Homotopy Perturbation Approach of Analyzing Diabetic Complications

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**ABSTRACT**

Diabetes is expanding over the world like a silent epidemic, posing a serious threat to public health. Diabetes modelling is an effective tool for tracking the increasing prevalence of diabetes and developing low-cost strategies for monitoring the medical disease and its repercussions. This article discusses a deterministic mathematical model for analysing diabetes complications, and the Modified Homotopy Perturbation method is used to solve the model equations. It became clear that the criteria used had a significant impact on the size of the diabetes population and the number of diabetics with complications.

**Keywords :** Diabetes Mellitus, Modified Homotopy Perturbation method, diabetic complications, mathematical model.

## INTRODUCTION

Diabetes Mellitus is simply the result of the body's failure to produce enough insulin to keep the sugar level stable[1]. Insulin injections are commonly recommended for those experiencing this type of physical failure. This is referred to as diabetes type I. Diabetes type II develops when the body rejects insulin. In addition to oral medication, this type of patient should follow a specific healthy eating plan and exercise programme to lose weight. However, heart disease is likely to affect these people in their later years [2]. Diabetes caused by gestation can occur briefly during pregnancy due to hormonal changes and usually appears in the fifth or sixth month of pregnancy (between the 24th and 28th week). Gestational diabetes usually resolves after the baby is born. However, 25-50% of women with gestational diabetes will develop diabetes later in life, especially if they need insulin during pregnancy or are overweight after childbirth [3].

Sharief and Sheta [3] improved diabetic detection by using a set of patient features to develop a mathematical model using the Multigene Symbolic Regression Genetic Programming technique. Genetic programming (GP) has showed significant advantages in constructing an evolving nonlinear model that may be used for prediction. The new GP model was evaluated using the PimaIndian dataset, and it demonstrated enhanced capability and accuracy in diabetes identification and treatment. Rosado [4] proposed a mathematical model to predict diabetes based on the 5-hour glucose intolerance test. Their model built on Ackerman's [5] idea, including three hormone concentrations rather than two. They included glucose and glucagon levels, as well as a global variable encompassing other hormones like insulin.The model was based on a 3x3 system of non-homogeneous ordinary differential equations. The system's coefficient parameters were computed using a nonlinear least squares method based on actual glucose tolerance test results. The simulations also produce an indicator similar to that proposed by Ackerman [5] for detecting a diabetic syndrome.

De Gaetano et al. [6] created a model of pancreatic islet compensation, presented its physiological assumptions, established some fundamental qualitative characteristics of its solutions, extensively discussed the numerical values assigned to its parameters (including cross-sectional epidemiologic data), and simulated its performance over a lifetime under various conditions, including worsening insulin resistance. The differences between two previously proposed diabetes progression models were noted, and the model was proposed as a realistic, robust explanation of the evolution of glucose-insulin system compensation in healthy and diabetic people. The basic concepts of Homotopy Perturbation Method (HPM), first proposed by Ji Huan [8,9], has been successfully applied to solve a wide range of linear and nonlinear functional equations. This method, which blends topological homotopy with conventional perturbation techniques, provides an easy way to obtain analytic or approximation solutions to a wide range of problems that emerge in several fields. The HPM he used to solve the Lighthill equation [8], the Duffing equation [10], and the Blasius equation [11] found its way into science and has been used to solve nonlinear wave equations [12], boundary value problems [13,14], quadratic Riccati differential equations [15], integral equations [16, 17, 18], Klein-Gordon and sine-Gordon equations [19, 20], initial value problems [21,22], Schrödinger equation [23], Emden- This broad range of applications highlights HPM's potential for solving functional problems. In this study, we built on Boutayeb's [7] work by conducting a mathematical analysis of diabetes and its consequences using the Modified Homotopy perturbation technique.

## The Mathematical Model

Boutayeb et al. [7] used ordinary differential equations and numerical approximations to measure the size of diabetic populations with and without complications. They investigated many options based on a set of factors, and the population's dynamic evolution from diabetes to diabetes with complications was clearly represented. Their methodology reveals how to accomplish efficient and cost-effective approaches by focusing on diabetes incidence and/or addressing complications as they develop.

According to Boutayeb et al. [7], the mathematical equation explaining the dynamics of diabetes and its complications is given by the following ordinary differential equations

$$\left.\begin{aligned} \frac{dD}{dt} &= I - (\lambda + \mu)D + \gamma C \\ \frac{dC}{dt} &= I + \lambda D - (\gamma + \mu + \nu + \delta)C \end{aligned}\right\} \quad (2.1)$$

$D = D(t)$represents the number of diabetics without complications.
$C = C(t)$represents the number of diabetics with complications.
$I = I(t)$denotes the incidence of DiabetesMellitus.
$\mu$ represents natural mortality rate.
$\lambda$ represents the probability of a diabetic person developing a complication.
$\gamma$ represents the rate at which compications are cured.
$\nu$ represents the rate at which diabetic patients with complication become severely disabled.

$\delta$ represents the mortality rate due to complications.
$N = N(t) = C(t) + D(t)$ denotes the size of population of diabetics at time $t$.
With $N = C + D$, we obtain

$$\left.\begin{aligned} \frac{dC}{dt} &= -(\lambda+\theta)C + \lambda N, t > 0 \\ \frac{dN}{dt} &= I - (\nu+\delta)C - \mu N \end{aligned}\right\} \quad (2.2)$$

where $\theta = \gamma + \mu + \nu + \delta$
with initial conditions

$C(0) = C_0, \quad N(0) = N_0 \quad (2.3)$

**Solution using Modified Homotopy Perturbation Method**

**Homotopy Perturbation Structure**
To illustrate the basic ideas of the method, He [9] considered the following nonlinear differential equation:

$A(u) - f(r) = 0, r \in \Omega \quad (3.1)$

subject to the boundary condition:

$B\left(u, \frac{du}{dn}\right) = 0, r \in \Gamma \quad (3.2)$

where $A$ is a general differential operator, $B$ is a boundary operator, $f(r)$ is a known analytical function and $\Gamma$ is the boundary of the domain $\Omega$. The operator $A$ can be divided into two parts $L$ and $N$, where $L$ is the linear part, and $N$ is the nonlinear component. Equation (3.1) may therefore be rewritten as:

$L(u) + N(u) - f(r) = 0, r \in \Omega (3.3)$

The homotopy perturbation structure is shown as follows

$H(v,p) = (1-p)[L(v) - L(u_0)] + p[A(v) - f(r)] = 0 \quad (3.4)$

where
$v(r,p): \Omega \to R \quad (3.5)$

In equation (3.4), $p \in [0,1]$ is an embedding parameter and $u_0$ is the first approximation that satisfies the boundary condition. It can be assumed that the solution of equation (3.4) can be written as power series as follows:

$v = v_0 + pv_1 + pv_2 + \cdots \quad (3.6)$
The best approximation for the solution is

$u = \lim_{p \to 1} v = v_0 + v_1 + v_2 + \cdots \quad (3.7)$

The series (3.6) is convergent for most cases; however, the convergence rate depends on the nonlinear operator $A(v)$ [9].

**Modified Homotopy Perturbation algorithm for finding the solution**

In this section, we present the algorithm of the new modification of the homotopy perturbation method [10-26]. To illustrate the basic ideas of the new modification, we consider the following nonlinear differential equation of fractional order:

$$D_*^{\alpha}u(t) + L\big(u(t)\big) + N\big(u(t)\big) = f(t), t > 0, m-1 < \alpha \le m, \quad (4.1)$$

where L is a linear operator which might include other fractional derivatives of order less than $\alpha$, $N$ is a nonlinear operator which also might include other fractional derivatives of order less than $\alpha, f$ is a known analytic function and $D_*^{\alpha}$ is the Caputo fractional derivative of order $\alpha$, subject to the initial conditions

$$u^k(0) = c_k, k = 0,1,2,\dots,m-1 \quad (4.2)$$

In view of the homotopy technique, we can construct the following homotopy:

$$u^{(m)} + L(u) - f(t) = p\big[u^{(m)} - N(u) - D_*^{\alpha}\big], p \in [0,1] \quad (4.3)$$

(Or)

$$u^{(m)} - f(t) = p\big[u^{(m)} - L(u) - N(u) - D_*^{\alpha}\big], p \in [0,1]\,. \quad (4.4)$$

The homotopy parameter $p$ always changes from zero to unity. In case p=0, Eqn (4.3) becomes the linearized equation

$$\frac{d^m u}{dt^m} + L(u) = f(t), \quad (4.5)$$

And Eqn(4.4) becomes the linearized equation

$$\frac{d^m u}{dt^m} = f(t) \quad (4.6)$$

And when it is one, Eqn (4.3) or (4.4) turns out to be the original fractional differential equation (4.1). The basic assumption is that the solution of Eqn (4.3) or (4.4) can be written as a power series in $p$

$$u = u_0 + pu_1 + p^2u_2 + \cdots \quad (4.7)$$

Substituting Eqn (4.7) into Eqn (4.3) or (4.4), and equating the terms with identical powers of $p$, we can obtain a series of linear equation of the form

$$p^0: \frac{d^m u_0}{dt^m} + L_0(u_0) = f(t), u^k(0) = c_k,$$
$$p^1: \frac{d^m u_1}{dt^m} + L_1(u_0, u_1) = \frac{d^m u_0}{dt^m} + N_0(u_0) - D_*^{\alpha}u_0, u^k(0) = 0,$$
$$p^2: \frac{d^m u_2}{dt^m} + L_2(u_0, u_1, u_2) = \frac{d^m u_1}{dt^m} + N_1(u_0, u_1) - D_*^{\alpha}u_1,$$
$$u^k(0) = 0,$$

.

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or the form

$$p^0: \frac{d^m u_0}{dt^m} = f(t), u^k(0) = c_k,$$
$$p^1: \frac{d^m u_1}{dt^m} = \frac{d^m u_0}{dt^m} + L_0(u_0) - N_0(u_0) - D_*^{\alpha}u_0, u^k(0) = 0,$$

$p^2: \frac{d^m u_2}{dt^m} = \frac{d^m u_1}{dt^m} - L_1(u_0, u_1) + N_1(u_0, u_1) - D_*^\alpha u_1,$
$u^k(0) = 0,$
.
.
.
respectively, where the terms $L_0, L_1, L_2, \ldots$ and $N_0, N_1, N_2, \ldots$ satisfy the following equations:

$$L(u_0 + pu_1 + p^2 u_2 + \cdots) = L_0(u_0) + pL_1(u_0, u_1) + p^2 L_2(u_0, u_1, u_2) + \cdots$$

$$N(u_0 + pu_1 + p^2 u_2 + \cdots) = N_0(u_0) + pN_1(u_0, u_1) + p^2 N_2(u_0, u_1, u_2) + \cdots$$

Setting p=1 in equation (4.7) yields the solution of (4.1). It is obvious that the linear equations in (2.8) or (2.9) are easy to solve, and the components $u_n,\ n \geq 0$ of the homotopy perturbation method can be completely determined, and the series solutions are thus entirely determined.

Finally, we approximate the solution $u(t) = \sum_{n=0}^{\infty} u_n(t)$ by the truncated series $\phi_N(t) = \sum_{n=0}^{N-1} u_n(t)$.
From (2.2)

$$\frac{dC}{dt} = -(\lambda + \theta)C + \lambda N \quad (4.8)$$
$$\frac{dN}{dt} = I - (\nu + \delta)C - \mu N \quad (4.9)$$

with initial conditions $C(0) = C_0, N(0) = N_0$.
Applying Modified Homotopy Perturbation to equation (4.8) and (4.9) with $q = \lambda + \theta,\ m = \nu + \delta$

$$\frac{dC}{dt} = -qC + \lambda N \quad (4.10)$$
$$\frac{dN}{dt} = I - mC - \mu N \quad (4.11)$$

The homotopy for the Eqns (4.10) and (4.11) are

$$\frac{dC}{dt} = p[\frac{dC}{dt} + qC - \lambda N - D^t C] \quad (4.12)$$
$$\frac{dN}{dt} = p[\frac{dN}{dt} - I + mC + \mu N - D^t N] \quad (4.13)$$

As above, the basic assumption is that the solutions of equations (4.12 & 4.13) can be written as
$C = C_0 + pC_1 + p^2 C_2 + \cdots \quad (4.14)$

$N = N_0 + pN_1 + p^2 N_2 + \cdots \quad (4.15)$

Therefore, by substituting Eqns (4.14 & 4.15) in (4.12 & 4.13), we get

$$\frac{d}{dt}[C_0 + pC_1 + p^2 C_2 + \cdots] = p[\frac{d}{dt}(C_0 + pC_1 + p^2 C_2 + \cdots) + q(C_0 + pC_1 + p^2 C_2 + \cdots) - \lambda(N_0 + pN_1 + p^2 N_2 + \cdots) - D^t(C_0 + pC_1 + p^2 C_2 + \cdots)]$$

$$\frac{d}{dt}[N_0 + pN_1 + p^2 N_2 + \cdots] = p[\frac{d}{dt}(N_0 + pN_1 + p^2 N_2 + \cdots) - I + m(C_0 + pC_1 + p^2 C_2 + \cdots) + \mu(N_0 + pN_1 + p^2 N_2 + \cdots) - D^t(N_0 + pN_1 + p^2 N_2 + \cdots)]$$

$\frac{dC_0}{dt} + p\frac{dC_1}{dt} + p^2\frac{dC_2}{dt} + \cdots = p\frac{dC_0}{dt} + p^2\frac{dC_1}{dt} + p^3\frac{dC_2}{dt} + \cdots + pqC_0 + p^2qC_1 + p^3qC_2 + \cdots - \lambda pN_0 - \lambda p^2N_1 - \lambda p^3N_2 - \cdots - pD^tC_0 - p^2D^tC_1 - p^3D^tC_2 - \cdots$

$$\frac{dN_0}{dt} + p\frac{dN_1}{dt} + p^2\frac{dN_2}{dt} + \cdots = p\frac{dN_0}{dt} + p^2\frac{dN_1}{dt} + p^3\frac{dN_2}{dt} + \cdots - Ip + mpC_0 + mp^2C_1 + mp^3C_2 + \cdots + p\mu N_0 + p^2\mu N_1 + p^3\mu N_2 + \cdots - pD^tN_0 - p^2D^tN_1 - p^3D^tN_2 - \cdots$$

with the initial conditions

$C(0) = C_0$
$N(0) = N_0$
and

$C_0 + pC_1 + p^2C_2 + \cdots = C_0$
$N_0 + pN_1 + p^2N_2 + \cdots = N_0.$

Equating the terms with identical powers of $p$, we obtain the following set of differential equations

$p^0 : \frac{dC_0}{dt} = 0 \quad , \quad C_0(0) = C_0$ (4.16)

$p^1 : \frac{dC_1}{dt} = \frac{dC_0}{dt} + qC_0 - \lambda N_0 - D^tC_0 = 0 \quad , \quad C_1(0) = 0$ (4.17)

$p^2 : \frac{dC_2}{dt} = \frac{dC_1}{dt} + qC_1 - \lambda N_1 - D^tC_1 = 0 \quad , \quad C_2(0) = 0$ (4.18)

.
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$p^0 : \frac{dN_0}{dt} = 0 \quad , \quad N_0(0) = N_0$ (4.19)

$p^1 : \frac{dN_1}{dt} = \frac{dN_0}{dt} + mC_0 - I + \mu N_0 - D^tN_0 = 0 , \quad N_1(0) = 0$ (4.20)

$p^2 : \frac{dN_2}{dt} = \frac{dN_1}{dt} + mC_1 + \mu N_1 - D^tN_1 = 0 , N_2(0) = 0$ (4.21)

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Consequently, the first few components of the homotopy perturbation solution for equations (4.16 & 4.19) are derived by integrating with respect to $t$, we get

$C_0(0) = C_0, N_0(t) = N_0$

$\frac{dC_1}{dt} = \frac{dC_0}{dt} + qC_0 - \lambda N_0 - D^tC_0$ (4.22)

$\frac{dN_1}{dt} = \frac{dN_0}{dt} + mC_0 - I + \mu N_0 - D^tN_0$ (4.23)

Integrating Eqns (4.22& 4.23) with respect to $t$, we get

$C_1(t) = C_0 - qC_0t + \lambda N_0t - C_0 + K_1$

At $t = 0, C_1(0) = 0 + K_1 = 0$

$\Rightarrow K_1 = 0$

which implies $C_1(t) = t(-qC_0 + \lambda N_0) = \alpha t$

where $\alpha = -qC_0 + \lambda N_0$.

$N_1(t) = N_0 + mC_0t - It + \mu N_0t - N_0 + K_2$

$= N_0 + (mC_0 - I + \mu N_0)t - N_0 + K_2.$

At $t = 0, N_1(0) = 0 + K_2 = 0$

$\Rightarrow K_2 = 0$

which implies $N_1(t) = t(mC_0 - I + \mu N_0) = \beta t,$

where $\beta = mC_0 - I + \mu N_0$

$$\frac{dC_2}{dt} = \frac{dC_1}{dt} + qC_1 - \lambda N_1 - D^t C_1 \quad (4.24)$$

$$\frac{dN_2}{dt} = \frac{dN_1}{dt} + mC_1 + \mu N_1 - D^t N_1 (4.25)$$

Integrating equations (4.24 & 4.25) with respect to $t$, we get

$$C_2 = q\alpha\frac{t^2}{2} - \lambda\beta\frac{t^2}{2} + K_3$$

At $t = 0, C_2(0) = 0 + K_3 = 0$

$\Rightarrow K_3 = 0$

which implies

$$C_2 = (q\alpha - \lambda\beta)\frac{t^2}{2}$$

$$N_2 = m\alpha\frac{t^2}{2} + \mu\beta\frac{t^2}{2} + K_4$$

At $t = 0, N_2(0) = 0 + K_4 = 0$

$\Rightarrow K_4 = 0$

which implies

$$N_2 = (m\alpha + \mu\beta)\frac{t^2}{2}$$

.

.

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and so on. In the same manner the rest of the components can be obtained. The $n^{th}$ term approximation solution is obtained as follows

$$C(t) = \sum_{i=0}^{\infty} C_i(t) = C_0(t) + C_1(t) + C_2(t) + \cdots$$

$$= C_0 + \alpha t + (q\alpha - \lambda\beta)\frac{t^2}{2} + \cdots$$

$$N(t) = \sum_{i=0}^{\infty} N_i(t) = N_0(t) + N_1(t) + N_2(t) + \cdots$$

## RESULTS AND DISCUSSIONS

Numerical simulations for different values are carried out and the results are outlined as follows:

Figures 4.1 and 4.2 depict the graphs of N(t) and C(t) versus t for various values of I. It was discovered that the number of diabetics increases quicker as the incidence of Diabetes Mellitus increases, but diabetics with complications drop faster as the incidence of Diabetes Mellitus declines.

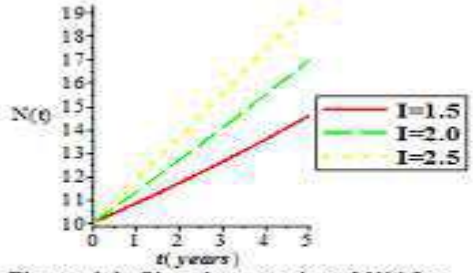


**Figure 4.1: Showing graphs of *N(t)* for different values of *I* when v=0.05, δ = 0.05, μ = 0.02, γ = 0.08, λ = 0.02,**

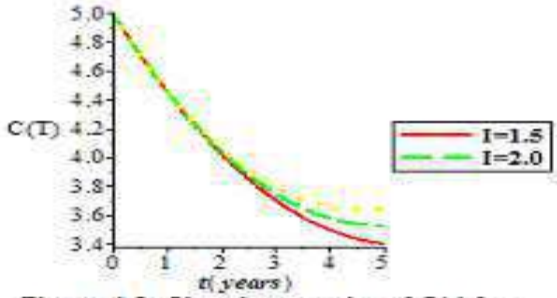


**Figure 4.2: Showing graphs of C(t) for different values of *I* when v=0.05, δ = 0.05, μ = 0.02, γ = 0.08, λ = 0.02,**

Figures 4.3 and 4.4 show graphs of N(t) and C(t) against t for various values of $v$. The number of diabetic patients with problems is increasing at a significantly higher rate. The likelihood of becoming seriously harmed increases. The number of diabetics with problems reduces quicker as the prevalence of Diabetes Mellitus raises.

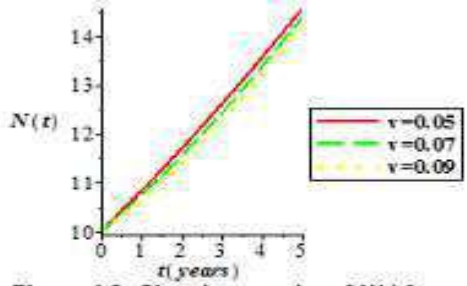


**Figure 4.3: Showing graphs of *N(t)* for different values of v when *I* = 1.5, δ = 0.05, μ = 0.02, γ = 0.08, λ = 0.02,**

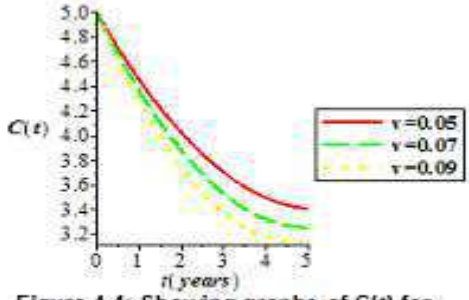


**Figure 4.4: Showing graphs of C(t) for different values of *v* when *I* = 1.5, δ = 0.05, μ = 0.02, γ = 0.08, λ = 0.02,**

Figures 4.5 and 4.6 exhibit the graphs of N(t) and C(t) against t for different values of $\delta$. Diabetics are becoming more prevalent as complications lead to lower mortality rates. Diabetics with concurrent disorders have a higher mortality rate.

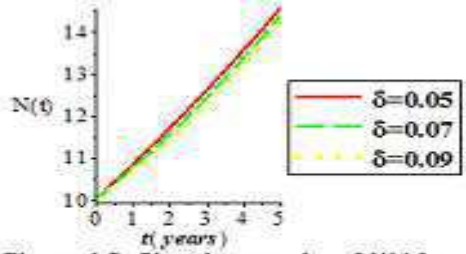


**Figure 4.5: Showing graphs of *N(t)* for different values of δ when *I* = 1.5, v = 0.05, μ = 0.02, γ = 0.08, λ = 0.02**

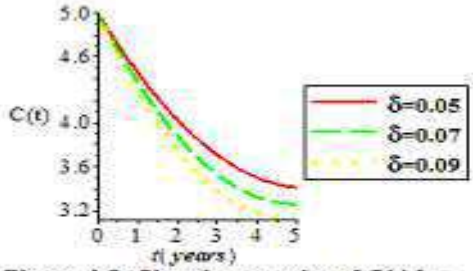


**Figure 4.6: Showing graphs of *C(t)* for different values of δ when *I* = 1.5, v = 0.05, μ = 0.02, γ = 0.08, λ = 0.02**

Figures 4.7 and 4.8 illustrate graphs of N(t) and C(t) against t for different μ values. As the natural lifespan decreases, N(t) increases more quickly, while C(t) decreases faster.

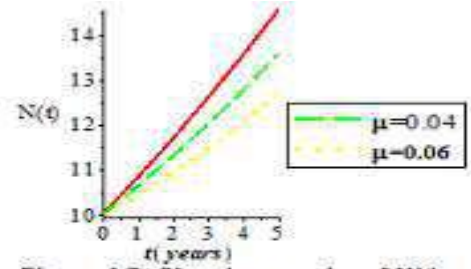


**Figure 4.7: Showing graphs of *N(t)* for different values of μ when *l* = 1.5, ν = 0.05, δ = 0.05, γ = 0.08, λ = 0.02**

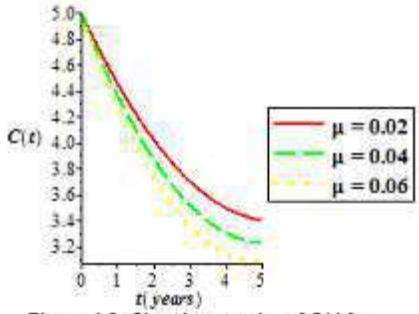


Figure 4.8: Showing graphs of *C(t)* for different values of μ when *l* = 1.5, ν = 0.05, δ = 0.05, γ = 0.08, λ = 0.02

Figures 4.9 and 4.10 show graphs of N(t) and C(t) versus t for various values of $\gamma$. It was discovered that the rate at which problems were resolved had no effect on either N(t) or C(t).

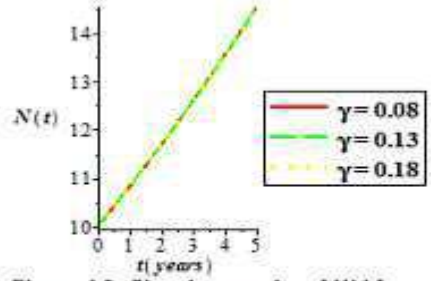


**Figure 4.9: Showing graphs of *N(t)* for different values of γ when *l* = 1.5, ν = 0.05, δ = 0.05, μ = 0.02, λ = 0.02**

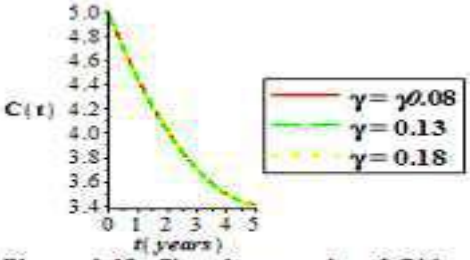


**Figure 4.10: Showing graphs of *C(t)* for different values of γ when *l* = 1.5, ν = 0.05, δ = 0.05, μ = 0.02, λ = 0.02**

Figure 4.11 presents the graph of N(t) against t for various values of $\lambda$. It was discovered that the rate at which problems were treated had no effect on N(t). Figure 4.12 displays the graph of C(t) against t for various values of $\lambda$. It was discovered that the number of diabetics with complications grows quicker when the likelihood of acquiring a complication increases.

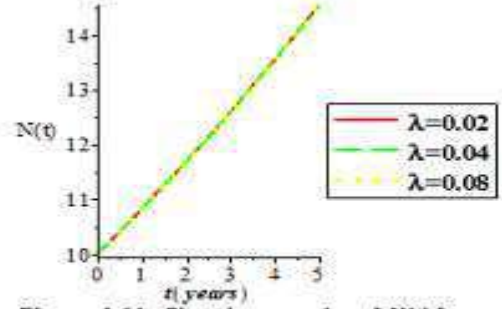


**Figure 4.11: Showing graphs of *N(t)* for different values of λ when *l* = 1.5, ν = 0.05, δ = 0.05, μ = 0.02, γ = 0.08**

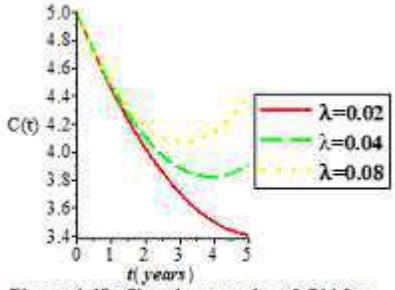


Figure 4.12: Showing graphs of *C(t)* for different values of λ when *l* = 1.5, ν = 0.05, δ = 0.05, μ = 0.02, γ = 0.08

## CONCLUSION

We explored an inevitable mathematical model of diabetes using the Modified Homotopy Perturbation Method, and the results demonstrate that the parameters involved played an important effect in the size of the diabetes population over

time and the number of diabetes with complications. This new algorithm is useful for the identification of complications which in turn helps to medicate the persons accordingly.

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**RESEARCH ARTICLE**

# Strategies for Ensuring Scalability and Fault Tolerance in Edge Computing Environments

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## ABSTRACT

Scalability and fault tolerance in edge computing environments can be achieved through a integration of strategies such as dynamic resource scaling, task offloading, redundancy, check pointing, and self-healing systems. By leveraging distributed computing principles, edge computing infrastructures can ensure reliability and high performance even in resource-constrained and failure-prone environments. The key is to adopt a combination of proactive metrics (example predictive maintenance) , reactive techniques (example failover mechanisms) to handle failures and resource limitations efficiently. Edge computing has emerged as a critical paradigm for enabling fly processing and minimizing delay in distributed applications like IoT, self-govern systems, and smart cities. However, ensuring scalability and fault tolerance in edge computing environments remains a significant challenge due to lack of the resource and decentralized architecture of edge nodes. This paper explores strategies for achieving scalability and fault tolerance in edge environments by leveraging dynamic resource scaling, hybrid cloud-edge architectures, and distributed edge orchestration. We also discuss the use of redundancy, replication, and failover mechanisms to enhance system reliability, along with advanced techniques like check pointing, self-healing, and predictive maintenance to recover from node failures. Through these techniques, edge computing systems can ensure high availability, seamless task execution, and robust system recovery, even in the presence of network disruptions or hardware failures. This paper highlights key

methodologies and technologies that enable scalable, resilient, and energy-efficient edge computing architectures, addressing the unique challenges posed by distributed edge environments.

**Keywords :** Scalability, Fault tolerance, Distributed systems, Dynamic resource scaling, Task offloading, Redundancy, Failover mechanisms, System recovery, Self-healing systems.

## INTRODUCTION

Edge computing environments bring computation and data storage closer to the devices that generate data, reducing latency and improving performance. However, ensuring scalability and fault tolerance in such distributed systems poses significant challenges due to resource constraints, network variability, and potential hardware failures. This paper outlines strategies for achieving scalability and fault tolerance in edge computing. Key techniques include the use of containerization and micro services to manage workloads dynamically, load balancing to optimize resource allocation, and decentralized architectures to minimize single points of failure. Fault tolerance is enhanced through redundancy, replication, and check pointing mechanisms, while auto scaling and predictive analytics help manage fluctuating demands. Additionally, leveraging hybrid cloud-edge models provides a seamless way to extend resources during peak loads. These strategies collectively ensure reliable and efficient edge computing systems capable of meeting diverse application requirements in dynamic and distributed environments.

The digital age is relentlessly pushing the boundaries of data processing, and edge computing is at the forefront of this revolution. It's a paradigm where computation happens near the data source instead of relying on a centralized cloud system. This approach not only reduces latency and bandwidth usage, but also allows for scalability and modularity, which are paramount in today's fast-evolving business landscape. Edge compute and storage needs to be scalable to accommodate different kinds of workloads and applications.

**Key Features of Edge Computing Architecture**

1. Decentralization: Unlike traditional cloud architectures, edge computing pushes data processing to the edge, decentralizing tasks to improve performance, reduce latency, and handle local events in real time.
2. Fast Response Time: Because the data remains localized , response times are significantly faster. This is critical for applications like autonomous systems, industrial automation, and real-time monitoring.
3. Throughput Enhancement : Edge computing reduces the volume of data that needs to be transmitted to the cloud. This not only conserves bandwidth but also reduces costs and improves efficiency.
4. Protection and Confidentiality: With edge computing, confidential information can be processed locally, reducing the risk of exposing it during transmission over the internet to a centralized data center. It also allows organizations to comply more easily with regulations like GDPR.
5. Intermittent Connectivity Support: Many edge deployments occur in regions with poor or inconsistent internet access. Edge nodes can operate independently and sync with the cloud when the connection is available, ensuring continuity of operations.
6. Real-Time Decision-Making: Edge nodes can process data and make decisions locally without having to wait for a response from a centralized cloud. This is crucial for applications demanding prompt response, such as robotic driving, emergency response systems.

**Edge Computing Architecture**

**Edge Computing Architecture Models**

**Device-Edge Model**

- Here, data is processed immediately upon the edge device itself, such as a Smartphone, wearable, or IoT sensor.
- This model is typically used in environments where data volumes are low, and real-time local decision-making is necessary.

**Local Edge Model**

- In this model, data is processed by nearby edge servers or gateways located on the premises (e.g., in a factory or retail store).
- The local edge model is ideal for environments that generate large volumes of data that must be processed quickly (e.g., in industrial IoT or video analytics).

**Regional Edge Model**

- Regional edge involves deploying larger edge data centers at strategic locations to serve a broader geographic area (e.g., a city or industrial park).
- This model is suitable for applications that require moderate latency, such as content delivery networks (CDNs) or regional data aggregation.

**Hybrid Edge-Cloud Model**

- A combination of edge and cloud, where edge nodes handle real-time, latency-sensitive tasks, while the cloud provides more powerful processing, storage, and long-term data analytics.
- This approach is common in AI-driven applications, where inference is performed at the edge, and the cloud handles model training.

**Challenges in Edge Computing Architecture**

- Scalability: Managing and scaling multiple edge nodes across different locations can be complex.
- Security: Decentralization introduces additional security challenges, such as ensuring data integrity across various edge devices.
- Standardization: Lack of standard protocols for edge devices and platforms may result in interoperability issues.
- Resource Constraints: Unlike cloud servers, edge devices often have limited compute, storage, and power resources.
- Scalability Strategies
- Distributed Architecture:
- Decentralized Control: Edge computing systems should use a decentralized or distributed architecture, where different edge nodes operate independently, processing data locally without relying on a central system. This minimizes bottlenecks and enables horizontal scaling as more devices or nodes are added.
- Micro services-Based Design: Deploy micro services at the edge. This allows applications to be divided into small, loosely coupled services that can scale independently as demand increases.
- Hierarchical Edge Layers: Deploy multiple layers of edge nodes, from smaller IoT devices to more powerful regional edge servers or micro data centers. This tiered approach can distribute the workload across different levels of the edge architecture, improving scalability.
- Edge Orchestration Platforms:
- Dynamic Resource Allocation: Use edge orchestration platforms to dynamically allocate resources (compute, storage, network) across edge nodes based on demand. Platforms like Kubernetes can orchestrate containerized applications, making it easier to scale services across multiple nodes.
- Workload Distribution: Distribute workloads intelligently across the edge network to avoid overloading a single node. Load balancing techniques can route requests to the least utilized or nearest node to enhance performance.
- Edge-Native Data Processing: refers to the practice of processing data directly at the edge of the network, close to the source of data generation, rather than relying on centralized data centers. This approach minimizes latency, enhances real-time analytics, and optimizes bandwidth usage by reducing the amount of data that needs to be

transmitted to the cloud. It is particularly beneficial for applications in IoT, autonomous systems, and real-time monitoring.

- Data Filtering and Aggregation: Process data at the edge to diminish the amount of information sent to the cloud. Edge devices can filter, Preliminary processing, and aggregate data before sending it to centralized systems, thereby reducing the burden on upstream servers and improving scalability.
- Federated Learning: In machine learning applications, train models locally on edge devices and aggregate the results at the central cloud. This approach allows scalability without moving large datasets back to the cloud for model training.
- Cloud-Edge Integration:
- Hybrid Cloud-Edge Architecture: Utilize the cloud to handle demanding tasks such as Archival storage, advanced analytics, or model training while offloading real-time processing to the edge. This hybrid approach ensures scalable resource use and flexibility across environments.
- Edge Offloading: In scenarios with heavy traffic or processing loads, offload excess processing tasks to more powerful edge nodes or regional data centers to handle high-demand periods.
- Containerization:
- Lightweight Containers: Using containerized applications ensures easy scaling and deployment. Containers provide flexibility, and tools like Docker and Kubernetes allow you to deploy applications across multiple edge nodes seamlessly.
- Server less Edge Computing: Implementing serverless functions at the edge can allow applications to scale rapidly based on demand, as resources are allocated dynamically without needing full server provisioning.

**Scalability Strategies**

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**Fault Tolerance Strategies**

**Redundancy and Replication**

- Edge Node Redundancy: Deploy multiple redundant edge nodes in strategic locations. If one node fails, another node can take over the workload, ensuring that services continue uninterrupted.
- Data Replication: Use distributed storage across edge nodes to replicate critical data. This ensures that if one node or data center goes offline, the data is still available from another location.

**Failover Mechanisms**

- Active-Passive Failover: In this setup, one edge node is active while another remains in standby mode. If the active node fails, the passive node automatically takes over, ensuring continued service availability.
- Active-Active Failover: Both nodes operate simultaneously, sharing the workload. If one node fails, the other node can take over the entire workload with minimal disruption.

**Edge Node Autonomy**

- Local Decision-Making: Ensure edge nodes can operate autonomously in the event of network disruptions or failures. Even if cloud connectivity is lost, edge nodes should continue to process data and perform critical tasks locally.
- Intermittent Connectivity Support: For deployments with unreliable connectivity (e.g., in remote locations), edge nodes should be capable of operating independently and syncing data with the cloud once the connection is restored.

**Load Balancing and Traffic Management:**

- Dynamic Load Balancing: Use load balancers to distribute incoming traffic across multiple edge nodes, avoiding overloading any single node and providing fault tolerance. If a node fails, the load balancer can redirect traffic to the remaining healthy nodes.
- Geo-Load Balancing: Spread workloads across geographically distributed edge nodes to ensure fault tolerance across regions. If an entire edge cluster fails, traffic can be directed to the next nearest edge cluster to maintain service availability.

**Edge Monitoring and Self-Healing**

- Real-Time Monitoring: Continuously monitor the health of edge nodes (CPU usage, network latency, memory consumption) to detect failures before they cause major disruptions. Tools like Prometheus or ELK Stack can help track system health and alert administrators to potential issues.

- Self-Healing Mechanisms: Implement automated self-healing mechanisms that restart services or reboot nodes if a failure is detected. This can be done through orchestration tools that ensure service continuity without manual intervention.

**Service Mesh for Edge**

- Resilience through Service Mesh: Implement service mesh architectures to handle inter-service communication and error handling. A service mesh can automatically reroute traffic between edge nodes in the event of node or service failures, providing resilience at the application layer.

**Edge-Specific Disaster Recovery (DR)**

- Geo-Redundancy: For critical edge deployments, data and services should be replicated across geographically dispersed edge nodes or micro data centers. In the event of natural disasters, geo-redundancy ensures that operations can continue from another location.
- Snapshot and Backup: Regularly take snapshots of the edge environment and store them either locally or in the cloud. These backups can be restored if an edge node or cluster experiences a major failure.

**Mesh Networking**

- Peer-to-Peer Communication: Deploy mesh networking protocols that allow edge nodes to communicate with each other directly. In the event of a network failure or node outage, mesh networking enables edge devices to find alternative routes for communication, enhancing fault tolerance.

**Security and Resilience**

- Edge Security Protocols: Implement robust security practices, including encryption, intrusion detection, and secure boot, to prevent malicious attacks that could cause edge nodes to fail.
- Zero Trust Architecture: Implement a zero-trust security model where every edge device and application must continuously verify its identity and access privileges. This reduces the likelihood of compromised edge nodes affecting the entire network.

## CONCLUSION

Scalability and fault tolerance are critical to the success of edge computing environments, given their distributed and resource-constrained nature. Implementing strategies such as containerization, micro services, decentralized architectures, and load balancing ensures efficient resource management and the ability to scale as demand fluctuates. Fault tolerance can be effectively enhanced through redundancy, replication, and check pointing, minimizing the impact of hardware failures or network disruptions. Moreover, integrating edge systems with cloud resources enables seamless scaling and redundancy, providing additional flexibility. By adopting these strategies, edge computing environments can become more robust, reliable, and capable of handling the demands of modern applications, especially in scenarios where low latency and high availability are essential

Scalability and fault tolerance are critical considerations for edge computing environments as they grow in size and complexity. Strategies like distributed architectures, dynamic resource allocation, load balancing, redundancy, and failover mechanisms are essential to ensuring that edge computing infrastructures can scale to meet increasing demands while maintaining resilience in the face of failures. Edge computing architecture is designed to complement Cloud computing enhances efficiency by situating processing and storage near the data source. It provides the low-latency, real-time processing necessary for next-gen applications, ensuring scalability and responsiveness across a distributed network of edge devices and nodes. With the growing adoption of IoT, 5G, and AI, edge computing will play an increasingly central role in various industries and use cases.

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**REVIEW ARTICLE**

# Retinal Vessel Segmentation: A Concise Review of Approaches and Obstacles

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## ABSTRACT

In automated retinal image processing, Retinal blood Vessels Segmentation (RVS) is a crucial step that helps with early detection and tracking of various types of eye diseases. In this paper, analyze eight different types of techniques are discussed namely, RVS-CRG-ML, RVS-HAN, RVS-VG-DDN, RVS-HIT, RVS-M-GAN, RVS-M-LAN, RVS-FA, and RVS-CK-MUM. It provides a brief but comprehensive review of the methods and difficulties in retinal vessel segmentation. After a brief overview to traditional methods, the narrative quickly shifts to the prominence of deep learning approaches, specifically Convolutional Neural Networks (CNN). The review addresses issues including inconsistent image quality, limited annotated datasets, and the interpretability of deep models while highlighting CNNs benefits in managing complex features. This work also discusses widely-used evaluation metrics and concludes with a few comments about potential future directions, such as the integration of multimodal imaging and developments in explainable AI. This concise review provides insights into the state and potential future paths of retinal blood vessels segmentation research, making it a useful tool for researchers and practitioners.

**Keywords:** Medical imaging, Retinal vessel segmentation, Image segmentation, Fundus image processing

## INTRODUCTION

The human eye [1] is a specialized sense organ that receives visual images and sends them to the brain.The rear surface of the eye, or fundus [2], is its interior. The retina, Optic Disc (OD), macula, fovea, and blood vessels include it. A specialized fundus camera is used in fundus photography to take images of the back of the eye by pointing through the pupil. With the use of these fundus images detect, monitor, and treat disease. The circular structure at the rear of the eye is known as the OD [3], or optic nerve head. Here is where the optic nerve and retina are connected. The major artery and vein of the retina enter the eye through the OD. The network of arteries and veins that carry blood to the retina the light-sensitive tissue located at the back of the eye is known as the retinal blood vessel [4] network. The retina is necessary for vision, and these veins are vital to preserving its health and functionality. Here, Figure 1 exposes the sample retinal fundus image and annotated retinal structure. For diagnostic and monitoring purposes, eye care practitioners frequently utilize imaging techniques including fundus photography [5], Fluorescein Angiography (FA), and Optical Coherence Tomography (OCT) to observe and study the retinal blood vessels. Diseases like Diabetic Retinopathy (DR), hypertensive retinopathy, retinal vein occlusion, and other vascular diseases that can affect vision are frequently related with abnormalities in the retinal vessels.

## SURVEY METHODS

This paper makes a survey with the followingeight papers in retinal vessel segmentation.

- RVS is based on Coupled Region Growing and Machine Learning approach (RVS-CRG-ML) [6]
- RVS is based on Hard Attention Net (RVS-HAN) [7]
- RVS is based on VG-DropDNet (RVS-VG-DDN) [8]
- RVS is based on Hessian based approach and Intensity Transformation approach (RVS-HIT) [9]
- RVS is based on M-GAN (RVS- M-GAN) [10]
- RVS based on Multi-Level Attention Network (RVS-M-LAN) [11]
- RVS based on Fluorescein Angiography (RVS-FA)[12]
- RVS based on Convolutional Kernels and Modified U-Net Model (RVS-CK-MUM) [13]

**Retinal vessel segmentation is based on Coupled Region Growing and Machine Learning approach (RVS-CRG-ML)**

Rodrigues *et al*.[6] establisheda multi-modal vessel segmentation framework known as ELEMENT (vEsseLsEgmentation using Machine lEarning and coNnecTivity). This framework consists of machine learningand region growth for pixel-based categorization together with feature extraction. Grey level and vessel connection attributes are the basis for the features, which are designed to gather complementing evidence. During the classification stage, the latter data is smoothly transmitted via the pixels.

The two primary phases of ELEMENT are:

- Region growth in coupled with machine learning-driven pixel classification,
- Feature extraction, which includes connection characteristics.

A manual per-pixel annotation method i.e., vessel or non-vessel is used to generate feature vectors from images containing ground truth and train the candidate classifiers. In the first stage, a predictive/classification model is created by training a classifier with ground truth and then stored for later use. To commence processing, the second step is to find one or more seed locations.

Retinal fundus images contain the first modality, which is represented by the well-known databases DRIVE, STARE, and CHASE-DB. The VAMPIRE dataset is utilized in the second modality, whichis a FA [15] retinal image. The IOSTAR and RC-SLO datasets are utilized in the third modality, Scanning Laser Ophthalmoscope (SLO) [16].

The foremost of this method is it used six datasets and another plus side of this method is it identified the thin vessels. The unworthy of this method is the time consumption is high.

**Retinal vessel segmentation is based on Hard Attention Net (RVS-HAN)**

Wang *et al.* [7] demonstrated the retinal vessel segmentation from the fundus images. Hard Attention net (HAnet) is an end-to-end deep learning[17]architecture designed for retinal blood vessels segmentation. Three decoder networks composed the design: the first one determines which image portions are "hard" or "easy" to analyze dynamically, and the other two seek to segment the retinal vessels in these "hard" and "easy" regions separately. HAnet has additional attention processes designed to strengthen the common high-level visual features found in vessel areas. In order to obtain the final segmentation result, the outputs from the decoders are finally combined and fed into a shallow network. In addition to two published color SLO [16] datasets, IOSTAR, RC-SLO and a self-collected small ICGA dataset, the network segmentation performance is evaluated on four benchmark fundus photography image datasets: DRIVE, STARE, CHASE_DB1 and HRF. In all the previously described datasets, HAnet obtains comparable performance when compared to other cutting edge conventional and deep learning based segmentation models. Additionally, HAnet's design is simply integrable with loss function adjustments and other baseline networks. The quality of this method is the HAnet is its ability to support training with various loss function adjustments due to the relative independence of its decoders. It fails to identify the tiny vessel which is the imperfection of this method.

**Retinal vessel segmentation is based on VG-DropDNet (RVS-VG-DDN)**

Desiani *et al*. [8]expressed Retinal blood vessels are segmented using the VG-DropDNet architecture. VG-DropDNet,is combined a dropout layer with VGG, DenseNet, and U-Net. A modified U-Net serves as a basis for the VG-DropDNet architecture.

The architecture is divided into three sections namely,

- Encoder
- Bridge
- Decoder components.

In order to obtain more features through deep networking, the encoder portion employed the VGG architecture. DenseNet was utilized on the bridge to call information from the previous layer, and dropout was mixed with it to prevent overfitting on DenseNet due to an excess of features from the concatenate operation. The Digital Retina Image for Vessel Extraction (DRIVE) and Retina Structured Analysis (STARE) datasets are processed using VG-DropDNet. The perfection of this work is VG-DropDNet is a strong, valid, and well-balanced model for segmenting blood vessels in retinal images. The precise and accurate blood vessels on retinal images[18] are provided by the Vg-DropDNet. The imperfection of this work is its only segments the retinal vessels it fails to identify the vessel disorders.

**Retinal vessel segmentation is based on Hessian based approach and Intensity Transformation approach (RVS-HIT)**

Alhussein *et al*. [9] introduced an unsupervised[19] method by combining aHessian-based method with anintensity transformation method. To improve the contrast of the retinal fundus images, here implemented with CLAHE [20]. For contextual region tuning of CLAHE, an improved version of the PSO algorithm is utilized. Wiener and morphological filtersare applied to the enhanced image to de-noise.To obtain the thick and thin vessel enhanced image and the intensity transformed image, respectively; the pre-processed image was exposed to separate use of the Hessian matrix method and the intensity transformation method. Apost-processing method was modified based on the related components region parameters obtained in the binary images. In order remove the non-vessel components from thick, thin, and intensity transformed binary images, the threshold values of the region parameters were empirically investigated and applied independently. The segmented binary imagine is obtained by applying the logical OR operation to the threshold images. The DRIVE and CHASE_DB1 datasets are utilized to evaluate the unsupervised method. The superior of this work is its segments very thin vessels. The worthiness of this work is time consumption is high.

**Retinal vessel segmentation is based on M-GAN (RVS- M-GAN)**

Park *et al*. [10]presented an accurate retinal blood vessel segmentation using the generative adversarial network known as M-GAN.The M-generator in the M-GAN utilizes the deep residual blocks, and the M-discriminator, which is a deeper network, effectively trains the adversarial model. A long-term residual connection is added by the M-generators, connecting each layer of the down-sampling network with the layer of the up-sampling network. The segmentation [21] and training efficiency are incorporated the binary cross-entropy loss function and the false-negative loss function. Specifically, the M-generator contains two-stacked deep FCNs that are constructed into the 'M' structure by copying and pasting the same network. DRIVE, HRF, CHASEDB1 and STARE databases are used for this M-GAN method.The supreme of this method is improved performance by applying the Lanczos resampling method for post-processing and the ACE algorithm for simple pre-processing. The useless of this work is it only segments the vessels it fails to identify the disease.

**Retinal vessel segmentation based on Multi-Level Attention Network (RVS-M-LAN)**

Yuan *et al*. [11] exposed to segment retinal vessels using the AACA-MLA-D-UNet. To reduce the over-fitting issue and preserve maximal vessel information between convolution layers, the dropout dense block is employed in place of the original convolution block. The adaptive atrous channel attention module is integrated into the contracting path to automatically sort the significance of each feature channel. The multi-level attention[22]module is includes three processes. For the purpose of creating the integrated multi-level feature, feature maps at various layers of the expanding path are first magnified and concatenated. Afterwards, an attentional feature module employed in the integrated multi-level feature refines the features at each individual layer. The enhanced feature maps are referred to as attentional features for ease of use. The final anticipated segmentation result is obtained by fusing the segmentation results produced by each attentional feature. DRIVE, STARE and CHASE databases are used for this analysis.The quality of this work is majority of vessels can be identified clearly from the background image. The unworthy of this method is it fails to directly identify the vessels and increase the vessel structures' connection

**Retinal vessel segmentation based onFluorescein Angiography (RVS-FA)**

Noh *et al*. [12] established a framework that uses corresponding Fluorescein Angiography (FA) images for registration and segmentation to achieve fine-scale vessel segmentation from fundus images. Here, used the fluorescent dye in the FA [15] frames to highlight the removed vessels, which we register and aggregate. Next, using an initial fundus vessel mask as a basis, this FA vessel mask is registered to the fundus image. The final vessel mask is refined through post-processing. The FA frames and FA vessel mask are registered to the fundus image using comparable coarse-to-fine hierarchical frameworks that include both projective and deformable registration. Both the fundus images and the FA frames' vessel segmentation are performed using two convolutional neural networks [14] with identical network topologies. SNUBH Fundus-FA, HRF, DRIVE, STARE and CHASE_DB1 datasets are used to train and test the images. The worth of this method is incolor retinal fundus images, the method generates accurate retinal vessel segmentation results that include filamentary vessels that are nearly invisible to the unaided eye.The unworthy of this method is,it contains some errors, and data needs to be expertly annotated in order to be classified as GT.

**Retinal Vessel Segmentation based on Convolutional Kernels and Modified U-Net Model (RVS-CK-MUM)**

Arias *et al*. [13] delivered an accurate and efficient deep learning-based vascular segmentation technique for images of the fundus of the eye. Here, the upscaling and downscaling stages, batch normalization and residual blocks are combined with a CNN that is built on a condensed version of the U-Net architecture. The network is trained using a unique loss function that takes into account each pixel's distance to the vascular tree after receiving patches that were taken from the original image as input. It provides the chance that every pixel in the input patch is a part of the vascular structure at its output. Applying the network to the patches that allow for the division of a retinal image yields the pixel-by-pixel probability map of the entire image. This method was successfully examined in the DRIVE, STARE, and CHASE_Db1 retinal image databases that were utilized for these analyses since they provide manual vascular structure segmentations. These segments are regarded as ground truth and enable the approaches to be evaluated in identical settings. The main metrics used to compare the algorithms' performance are Acc and AUC,

which measure the algorithms' ability to segment vascular trees at a certain operating point and over the full range of potential work points, respectively. This method operates with the highest level of accuracy over the entire set of feasible operation point which is the quality of this work. The failure of this work is it tested only very less amount of images.

## DISCUSSION AND ANALYSIS

This survey uses analysis metrics such as segmentation accuracy to examine retinal vessel segmentation methods including RVS-CRG-ML, RVS-HAN, RVS-VG-DDN, RVS-HIT, RVS-M-GAN, RVS-M-LAN, RVS-FA, and RVS-CK-MUM. Table 1 presents an analysis based on general properties, Table 2 delves into a merits and demerits analysis, and Table 3 exhibits an analysis based on performance measurement. The accuracy analysis for image segmentation is finally shown in Figure 2.

## CONCLUSION

The retinal vessel segmentation is essential for diagnosing and treating systemic and ocular diseases. This review has covered several types of methodologies namely, RVS-CRG-ML, RVS-HAN, RVS-VG-DDN, RVS-HIT, RVS-M-GAN, RVS-M-LAN, RVS-FA, and RVS-CK-MUM each with unique benefits and drawbacks, from advanced deep learning method to more conventional image processing techniques. Conventional method are more straightforward but have trouble processing complicated images, deep learning methods are more accurate but need a lot of computational resources and annotated data. In order to overcome these obstacles, future research should investigate multimodal data integration, semi-supervised and unsupervised learning methods, and domain adaptation methods.

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**Table 1: Analysis based on general properties**

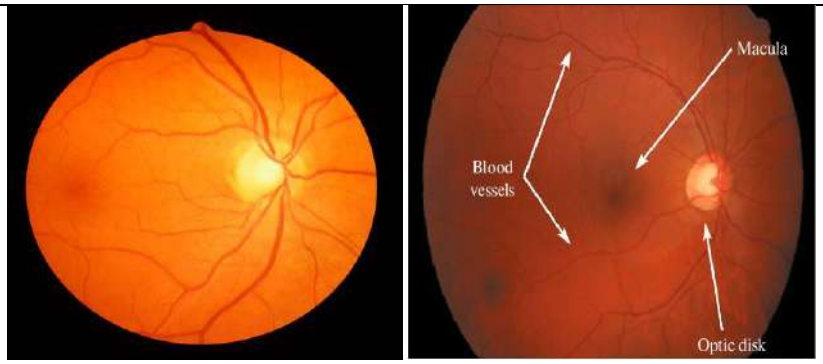
| Method | Journal | Publication | Year | Input image | Segmentation method |
|---|---|---|---|---|---|
| RVS-CRG-ML [6] | IEEE | Journal of Biomedical and Health Informatics | 2020 | Gray scale | ELEMENT |
| RVS-HAN [7] | IEEE | Journal of Biomedical and Health Informatics | 2020 | Gray scale | HardAttention net (HAnet) |
| RVS-VG-DDN [8] | IEEE | IEEE Access | 2022 | Color image | VG-DropDNet |
| RVS-HIT [9] | IEEE | IEEE Access | 2020 | Gray scale & Color image | Unsupervised |
| RVS-M-GAN [10] | IEEE | IEEE Access | 2020 | Color image | M-GAN |
| RVS-M-LAN [11] | IEEE | Journal of Biomedical and Health Informatics | 2022 | Color image | AACA-MLA-D-UNet |
| RVS-FA[12] | IEEE | IEEE Access | 2020 | Gray scale & Color image | Multimodal registration |
| RVS-CK-MUM [13] | Elsevier | Computer Methods and Programs in Biomedicine | 2021 | Gray scale & Color image | Convolutional Neural Network |

**Table 2: Analysis on the merits and demerits**

| Method | Merits | Demerits |
|---|---|---|
| RVS-CRG-ML | Identified the thin vessels | Time consumption is high |
| RVS-HAN | HAnet is its ability to support training with various loss function adjustments due to the relative independence of its decoders | Fails to identify the tiny vessel |
| RVS-VG-DDN | Strong, valid, and well-balanced model | Fails to identify the vessel disorders. |
| RVS-HIT | Segments very thin vessels | Time consumption is high. |
| RVS-M-GAN | Improved performance by applying the Lanczos resampling and the ACE algorithm | Fails to recognize the illness. |
| RVS-M-LAN | Majority of vessels can be identified clearly from the background image | Fails to directly identify the vessels and increase the vessel structures' connection |
| RVS-FA | Generates accurate RVS results that include filamentary vessels that are nearly invisible to the unaided eye | Data needs to be expertly annotated in order to be classified as GT |
| RVS-CK-MUM | Highest level of accuracy | Tested only very less amount of images |

**Table 3: Analysis based onperformance measurement**

| Author Name | Segmentation Method | Accuracy (%) | No. of database used | Database name |
|---|---|---|---|---|
| Rodrigues *et al.* | RVS-CRG-ML | 82% | 6 | DRIVE, STARE, CHASE-DB, VAMPIRE, IOSTAR and RC-SLO |
| Wang *et al.* | RVS-HAN | 83% | 4 | DRIVE, STARE, CHASE_DB1 and HRF |
| Desiani *et al.* | RVS-VG-DDN | 89% | 2 | DRIVE and STARE |
| Alhussein *et al.* | RVS-HIT | 83% | 2 | DRIVE and CHASE_DB1 |
| Park *et al.* | RVS-M-GAN | 85% | 4 | DRIVE, HRF, CHASEDB1 and STARE |
| Yuan *et al.* | RVS-M-LAN | 90% | 3 | DRIVE, STARE and CHASEDB |
| Noh *et al.* | RVS-FA | 85% | 5 | SNUBH Fundus-FA, HRF, DRIVE, STARE and CHASE_DB1 |
| Arias *et al.* | RVS-CK-MUM | 87% | 3 | DRIVE, STARE, and CHASE_DB1 |




**(a) (b)**

**Fig. 1.a) Sample retinal Fundus image and b) Annotated retinal structure**

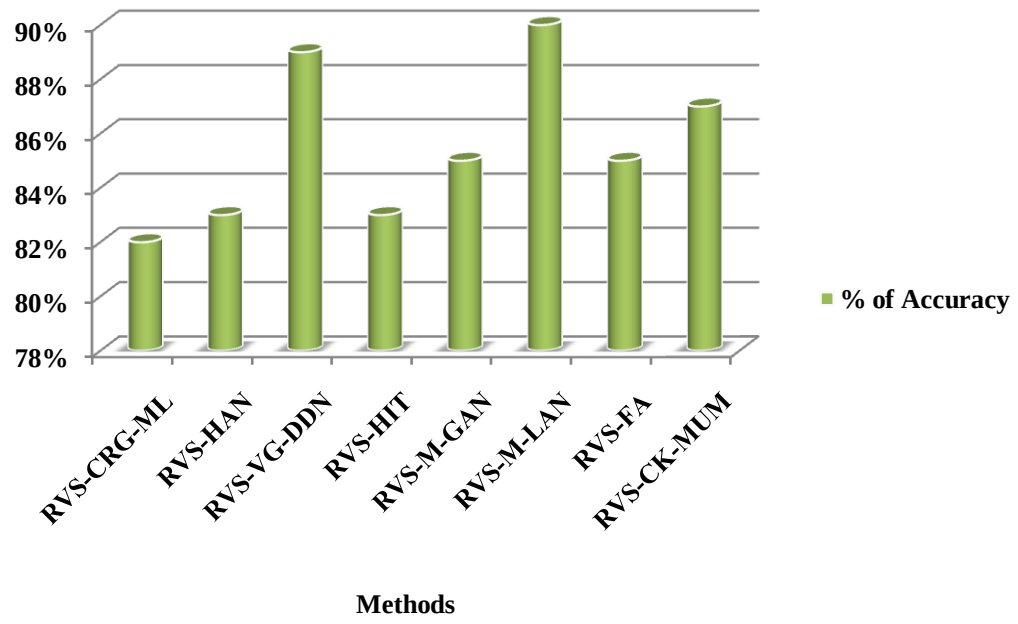



**Fig. 2. Accuracy analysis for image segmentation**

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**RESEARCH ARTICLE**

# Survey on Future Bill Prediction System in Electricity Consumption

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**ABSTRACT**

The Digital world is emerging out. Digital devices, networks, and power equipment are required in our current scenario, as well as in comparison to our pandemic situation. To make plans for energy savings, it is critical to understand the electricity bill and its components. All of the digital transformations necessitate a significant amount of electricity consumption. According to some states, the generation of electricity bills occurs once every two months. Our electricity bills contain a lot of information that can provide us with a good understanding of our electricity consumption patterns. A thorough understanding of the various components can aid in the planning of a cost-cutting exercise. Most of the moderate salaried people dealing with electricity bills as a major burden. Due to devices and usage of power consumptions are increasing the power demand also increasing, this may lead to an unmanageable situation. So the Smart meter, power monitoring, predicting the bill for the future as well as the current Monthly bill is an essential thing. By Tracking the power consumption wastage of power consumption, overload of electrical Devices, balanced electricity bill can be obtained. The tracking of power Consumptions accompanied by Smart meters and IoT devices incorporated. There are two sorts of energy screens: entire house energy screens and handheld energy screens that you can take anywhere in your home. The electricity consumption speaks with a little isolated unit that clasps onto your power meter and shows constant information on how much power you're utilizing.

**Keywords:** electricity bill, devices, power consumption, smart meter, power monitoring, predicting the bill,

## INTRODUCTION

Huge information investigation portrays the most common way of revealing patterns, examples, and relationships in a lot of crude information to assist with settling on information-educated choices. These cycles utilize natural factual

investigation strategies—like bunching and relapse—and apply them to more broad datasets with the assistance of fresher instruments. Enormous information has been a popular expression since the mid-2000s when programming and equipment capacities made it workable for associations to deal with a lot of unstructured information. With the impact of data, early improvement ventures like Hadoop, Start, and NoSQL information sets were made for the capacity and dealing with of huge data. This field keeps on advancing asinformation engineers search for approaches to incorporate the tremendous measures of complex data made by sensors, organizations, exchanges, keen gadgets, web utilization, and that's only the tip of the iceberg. Indeed, even presently, huge information examination techniques are being utilized with arising innovations, similar to AI, to find and scale more mind-boggling experiences.

Power utilization in Indian homes has significantly increased since 2 decades. The Level of admittance of the families consuming the power has been extended from 55% to 80% .This statistics is been calculated between 2001 and 2017.On considering year 2014 the family accommodate on India which is nearly 90 units of electricity as per monthly consumption. It is normal to have gadgets like four number of fans, small screen, and 1.5 litre capacity of Cooler box. This is three-fourths of the conventional month-to-month family use in China, a 10th of that in the USA, and 33% of the world common

**Three insights emerge:**
At last, the power utilization inside states additionally displays a huge imbalance at the family level. As per the National Sample Survey Office (NSSO's) overviews, about 20% of charged families devour under 30 units of power each month, while about 80% burn-through under 100 units each month. In rustic regions, 90% of the electric families devour under 100 units. This appropriation differs with states. In many states, around 15-20% of the multitude of families devour under 30 units each month. The states devouring the least power are Karnataka, West Bengal, Bihar, and Jharkhand. For additional subtleties on outcomes see our new report.

Tangedco is set to sign an MoU with Energy Efficiency Services Limited (EESL) to secure 10 lakh brilliant meters. These shrewd meters will be introduced on all premises that devour in excess of200 units of force each month in a staged way from June 1. As of now, computerized meters have been introduced at almost 80% of purchaser premises and these will be supplanted by brilliant meters. A savvy, mechanized metering framework decreases meter-perusing, information passage blunders, and expenses by eliminating the requirement for manual meter perusing. The brilliant meter can empower utilities to lessen their total transmission and business (AT&C) misfortunes and charging failures. These meters, related through an internet noticing system, will help with reducing business adversities of utilities, overhaul wages, and fill in as a basic gadget in impact region changes. Most electric utilities serve a doled-out geographic area, by and large without various competitors moving toward their customers. In light of everything, utility expenses have routinely been set by neighbourhood, state, or government regulators, components that study electric utility costs, salaries, adventure decisions, fuel costs, and various segments to appear at a target speed of return. Five vital components of your power bill:

- Power metering
- Request
- Energy utilization
- Burden factor
- Force factor

## LITERATURE REVIEW

Understanding the impact of purchaser's consideration on their power use lead and charged by the shortage of BDA research that splendid lights on the purchaser's side of the energy district. Utilizing illustrative and insightful assessment on the mined client's information, the model can show, portray and foresee power use of a specific family, given it's a profile. Evaluation comes about appeared that the show has the basic parts in giving

the crucial information to assist the clients track and screen their control utilize. It can in like way offer assistance them with engineering Keeping up the Astuteness of the Details

The layers are information sources, information pre-arranging, information assessment, and information assignment centre and information show. The principle layer is data arrangement from both AMI (Advanced Metering Infrastructure) and Customers informational indexes. The customer informational collection involves coordinated data and AMI contains both coordinated and unstructured data. This will convert the data into the needed format for analysing purposes. Then Data cleansing and analysis need to be done with data analytics using both Descriptive and Predictive analysis. Now the processed data underwent to Data warehouse. The most used information distribution centre for enormous information execution is Hadoop disseminated document framework (HDFS). HDFS have enormous scope datasets across various hubs repetitively to handle failover and flawed occasions. A non-social data set. Openings for the model execution of the BDA that is focusing on the purchaser side of the energy region are abundant. The model, which utilizes clear and prescriptive examination targets furnishing the customers with the examination results that can help them to more readily deal with their family power utilization. Taking into account the evaluation performed, it tends to be induced that the proposed BDA model for family power use following and noticing is viewed as usable and prepared to achieve its objective et al. [1] [2].

In this paper, they planned one DSS Framework that works inside an IoT biological system. This gives a progressed investigation of Smart meter for upgrading the nature of information works on the expense forecast, utilization and furthermore that incorporates choice with noteworthy suggestions. The dataset has been taken from the Commercial organization. The methodology given here is with Bayesian organization alongside three AI classifiers for expectation as Random backwoods, Naïve Bayes, and choice tree. Results display that our procedure delivers quantifiably basic evaluations and that the DSS will chip away to the detriment capability of ESM network exercises and Support. Accuracy assesses the limit of explicit classifiers to accurately arrange unlabelled data. It presents the extent between the different definitely requested data (for both misguided and write requests) and the amount of gave data. Preliminaries performed on the dataset showed the efficiency and the adequacy of the proposed approach [2].

[3] The Geographically distributed data centres serve as an armature for Cloud services. Which consumes a huge amount of electricity this leads to huge costs for operational services and it is a typical challenge for cloud computing. On the off chance that the power costs of server farms are anticipated ahead of time, the cloud supplier can diminish energy costs. An effective power value expectation is required for limiting the power bill of GDCs. This paper proposes power value expectations for GDCs in multi-district power markets. The test is led on genuine power value informational collections with AI calculations. By relatively surveying the forecast precision of the models, the most exact one is chosen. Analysis results show that the forecast model can give promising precision. They presented the electricity bill prediction for nearly three sectors in the United States using some Machine Learning algorithm. This consists of the dataset for three years in each sector. It produces accurate results comparing to the decision table and linear regressions. The analyses express the viability and execution accomplished by the chose models considered for both ML calculation and yearly prepared informational index. They chose models to give promising precision to foreseeing the power cost of GDCs in multi-area power markets. The models can't foresee well the value spikes and instability. Later on, enhancements to the models, for example, the issue of Predictability in the cost will be tended to.

[4] Due to the huge extension of cities, and industrial, commercial growth the energy consumption is in high demand. Because of IoT and smart meters the energy metering is possible in all places. For forecasting purposes, the Machine Learning algorithm will be used. RNN (Recurrent Neural network) this paper utilizes Recurrent Neural Networks (RNN) to catch time conditions and proposes an original energy load determining system based For example age and Sequence-to-Sequence (S2S) profound learning calculation. The S2S design that is regularly utilized for language interpretation was adjusted for energy load determining. Analyses centre around Gated Recurrent Unit (GRU) based S2S models and Long Short-Term Memory (LSTM) based S2S models.

[5] Inthis paper, the author proposed a deep learning prediction method that considers the weather and the progressive rate. In this paper, they propose a deep learning-based hypothetical system for environmentally friendly single-family energy rates. They used the weighted mean square error to set up the proposed model for better accuracy. Finally, according to the proposed model, the expected energy consumption is converted into monthly energy charges.

[6] The consistently growing interest for power has driven various standard resources almost utilization. The danger presently exists over limitless wellsprings of power that haven't been developed to the point where they can fully function independently. Ideal sharing of power and usage of particular energy are called into action in such a scenario. The proposed framework here fulfills the need of ceaselessly recognizing energy use and alarming the purchaser when it surpasses a foreordained line. This kind of use, which is made nearby a stuff module imagined iSocket It is utilized to follow the measure of energy devoured by every device in a home and therefore creates a month to month power bill. As of now, in this framework, the customer can build up a month to month required bill, and as energy utilization surpasses the put forward line, the shopper is educated through the thing application. Also, this framework utilizes a calculation that sets the seeing the speed of a connected contraption to the association is dependent depending on the type of gadget and, as a result, is an energy-saving gadget. The ACS712 current sensor was used in this piece, and the microcontroller used to operate the stuff units was an Arduino UNO et al. [6] the force supply to recognising contraptions and regulators is provided by a boost converter. The utility bill checker programme is distributed. It gives presumptions for things to be utilized at an unequivocal time span dependent upon its force utilization.

In this paper, a hardware module has been generated, which is used to check the amount of energy consumed by each contraption in a house and, as a result, compares the month to the monthly power bill. This framework utilizes a calculation that sets the seeing speed of a connected contraption to the association is dependent upon the kind of gadget and subsequently. The ACS712 current sensor was used in this piece, and the microcontroller used to operate the stuff units was also an Arduino Ide. The force supply to the differentiating contraptions and regulators, among many other things, is furnished by a boost converter. [6] The application is sent to verify electric bills. The framework takes the commitment of relevant data and forecasts grid inspections for the upcoming months. It offers presumptions for objects to be used over an apparent timeframe based on its force usage and other factors. [6]

[9] This is to analyse the impact on consumption of electricity based on smart homes, here the Prediction Model. According to this paper the Data related to Energy consumption is from smart homes along with devices, home appliances that can be monitored and automatically controlled. Next one is General content from smart grid which have integrated communication information and also computational power. The data has been used for three terms as short, middle, long term prediction. This kind of prediction leads to optimal functioning of energy consumption. The data for energy related present in non-linear pattern. The changing weather, climate condition, population, agile seasonal environment. The Ml based prediction includes Artificial/Recurrent Neural Network, Feed forward, Wavelet Transform, Modified artificial bee colony.[9]

**TECHNIQUES USED**

**Naïve Bayes:**

Naive Bayes classifiers are a combination of collection estimations subject to Bayes' Theorem. It's everything except a single estimation anyway a gathering of computations where all of them shares a commonplace standard, for instance, each pair of arrangements being assembled is liberated from each other. This is a classifier used to track down the precise degree of expectations.

**Random Forest**

An irregular backwoods is a Meta assessor that fits various choice tree classifiers on different sub-examples of the dataset and utilizations accrediting to work on the prescient exactness and authority over-fitting. The sub-example

size is controlled with the max_samples boundary if bootstrap=True (default), in any case, the entire dataset is utilized to construct each tree. This is also one type of classifier used along with Naïve Bayes classifiers.

**Decision Tree**

The indecision tree, the information goal play the main role. Which makes the information goal as a root node. A decision tree is a most powerful tool and acts as a flow chart. The branches in Decision trees are like branches in the normal tree the prediction becomes easy and it is a classical representation of values either in weighted or non-weighted format.

**Simple Moving Average**

This is one of the method used for Forecasting purpose. The working principle of this method is which will calculate the distance moved from one place to another place that is from source to destination and take that particular value as interval value and place it in some variable named x, Now the particular x value is used for forecasting purpose. So this method can also supports the forecasting of electricity bill by just with in certain interval of time it find out the cost variation , leads to forecasting electricity bills. This simple moving average mainly used for graphical presentation. It leads to good results

**Neural Network**

Which can able to solve the complicated as well as difficult data to its strength. Mostly it is used in pattern recognition, data manipulations. The main strength of Neural Network is fault tolerance. Most probably it is used for prediction, validation and also research. So while moving on to data collected for new and old electricity consumption charges has been complicated and be difficult to handle. So the neural network will come up with this neural network techniques. This one of top techniques used for prediction for future data analysis, not only in particular field but also in different fields.

**ANALYTICAL MODELS FOR PREDICTION**

A. Classification Model:

It is one of the simplest model for prediction analysis, working principle for this classification model mostly based on yes or no question and categorization will be undergone. From the name itself the classification model is a model which is used to classify the data or needs as per our requirements.

**B. Forecast Model**

This is also one of the prediction analysis model, which is also based on the historical data can be collected based on real time dataset or non realtime dataset. It is largely using prediction Model. Which deals with metric value that means for example at Electricity bill the amount per unit will vary based on different criterias like one unit will cost so and so amount per unit until it doesnotexeeds 500 units. The unit per cost will be vary from 501 to 1000 units and so on .

**Time Series Model**

The time series models is also used for predicting the future weeks or months. The data for this prediction model has been collected in the form of timely manner as input data become time. Based on the old data it will predict the next upcoming predictions.

**Electricity bill visualization**

By means of conumer Electricity bill consumption the data for particular month and data for the past before Pandemic situation has been considered. The average power consumption before . The above Fig.1. Represents the consumption of electricity after Pandemic situation. X-axis represents the customers and y axis represents the no of units consumed. Fig.2. represents the electricity consumption before pandemic situation. The deviation of unit consumption due to two criteria, first one is work from home, the second one is vacated to home town. The data for

the above analysis like electricity bill consumption before and after pandemic situation has been taken from the Tamil Nadu Electricity Board.

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**Table 1. Electricity bill visualization:**

| sno | meterno | Month and year | units | bills | U |
|---|---|---|---|---|---|
| 1 | 9264660510 | October and 2021 | 740 | 3314.0 | |
| 2 | 9264674716 | October and 2021 | 440 | 440.0 | |
| 3 | 926467439 | October and 2021 | 660 | 2786.0 | |
| 4 | 9264660274 | October and 2021 | 630 | 2588.0 | |
| 5 | 9264674728 | October and 2021 | 360 | 680.0 | |
| 6 | 926467438 | October and 2021 | 377 | 630.0 | |
| 7 | 7064008498 | October and 2021 | 630 | 2588.0 | |
| 8 | 9264660373 | October and 2021 | 150 | 75.0 | |
| 9 | 9264660456 | October and 2021 | 200 | 150.0 | |
| 10 | 9264660457 | October and 2021 | 190 | 135.0 | |
| 11 | 9264660473 | October and 2021 | 230 | 290.0 | |
| 12 | 9264660474 | October and 2021 | 740 | 3314.0 | |
| 13 | 9264660476 | October and 2021 | 562 | 5162.0 | |
| 14 | 9264674331 | October and 2021 | 90 | 0.0 | |
| 15 | 9264674545 | October and 2021 | 679 | 2911.4 | |
| 16 | 95680211534 | October and 2021 | 150 | 75.0 | |
| 17 | 7016907654 | October and 2021 | 200 | 150.0 | |
| 18 | 2578231120 | October and 2021 | 630 | 2588.0 | |
| 19 | 92300021325 | October and 2021 | 150 | 75.0 | |
| 20 | 5156015494 | October and 2021 | 230 | 290.0 | |
| 21 | 4457006909 | October and 2021 | 450 | 980.0 | |

**Fig. 1. Consumption of electricity after pandemic situation**

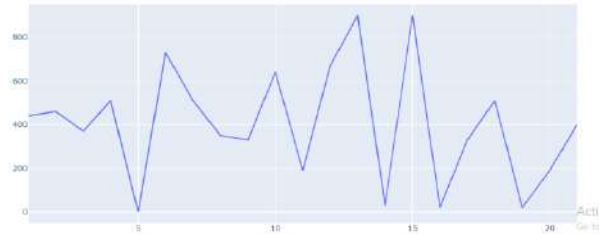

**Fig. 2. Consumption of electricity before pandemic situation**

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**RESEARCH ARTICLE**

# Identification of Human Diseases based on Symptoms using an Ensemble Machine Learning Algorithm

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## ABSTRACT

In this study, we developed a machine learning pipeline to classify diseases based on symptom inputs using three distinct classifiers: Support Vector Machine (SVM), Random Forest (RF) and Naive Bayes (NB). The pipeline incorporates data preprocessing, model training, evaluation, and an ensemble prediction approach. Firstly, the dataset has been pre-processed by loading it, missing values handling, and encoding the target variable. Further, the dataset is split into training set and testing sets. The models were evaluated using cross-validation to ensure robust performance estimates. The three trained classifiers are SVM, NB, and RF, and evaluated their performance on both training and testing datasets. The results showed competitive accuracy across all models, with each classifier demonstrating strengths in different areas. Confusion matrices were plotted for visualizing the performance of each model. To further improve the prediction accuracy, Combined the predictions from the three models using a voting mechanism. This ensemble approach aggregates the predictions by taking the mode of the individual predictions, which enhanced the overall accuracy. Additionally, symptom index is created and developed a function to predict diseases based on given symptoms. This function leverages the trained ensemble model to provide a final prediction along with individual model predictions. Overall, this pipeline demonstrates an effective method for disease classification using an ensemble of machine learning models, providing a robust tool for medical diagnosis based on symptoms.

**Keywords:** Symptom-Based Prediction, Random Forest (RF), Naive Bayes (NB), Ensemble Learning, Healthcare Diagnosis, Confusion matrices, Data Preprocessing, and Model Evaluation.

## INTRODUCTION

The usage of machine learning (ML) algorithm in healthcare is expanding swiftly, driven by the demand for predictive, personalized, and cost-effective care. Disease prediction is crucial for timely diagnoses, which can lead to life-saving interventions and reduced treatment costs. According to the World Health Organization, the early identification oghuman diseases such as cancer, heart conditions, and chronic ailments can notably increase survival rates. ML models can analyze intricate datasets from electronic health records (EHR), genetic information, and patient histories to predict disease risk, laying the groundwork for precision medicine. Machine learning (ML) algorithm is one of the widely adopted methodology for disease prediction due to its ability to analyze complex datasets and uncover hidden patterns, which are often not evident through traditional statistical methods. Machine learning facilitates early disease detection by examining patterns and risk factors in patient data. Through tailoring treatments to an individual's genetic makeup and lifestyle, machine learning supports a transition from one-size-fits-all healthcare. ML models can aid healthcare providers in efficiently allocating resources by identifying high-risk patients requiring immediate attention. This phase involves preparing, encoding, and converting medical datasets into a suitable format for machine learning models. Managing missing values, standardizing features, and encoding categorical variables are crucial stages. Extracting features like patient demographics, lifestyle elements, and symptoms to improve model accuracy. This study explores three main classifiers Support Vector Machine (SVM), Effective for classifying high-dimensional data. Naive Bayes (NB) algorithm Utilizes Bayes' theorem and is well-suited for probabilistic disease predictions. Random Forest (RF) is an ensemble technique that combines multiple kinds of decision trees to enhance an accuracy. Evaluation Metrics such as accuracy, recall, precision, and F1-score aid in assessing the model's performance. The cross-validation technique called k-fold validation is used to ensure the generalizability of the model across new data.

## REVIEW OF EXISTING LITERATURE

In a study by Sonar & Jayamalini (2019), Support Vector Machine (SVM) and the Logistic Regression were utilized to predict diabetes, yielding an accuracy of 87.4%. This research underscored the importance of feature selection in enhancing model performance, as the selection of the right features improved accuracy and computational efficiency. Similarly, Fawaz *et al*. (2020) used Recurrent Neural Networks (RNNs) to forecast diabetes based on temporal glucose data, achieving an 89% accuracy. Their work emphasized the significance of incorporating time-series data for predicting chronic diseases to capture temporal trends in patient health records. Archana & Kumar (2020) employed Random Forest and Naive Bayes algorithms for heart disease prediction, achieving an accuracy of 89.6%. Their use of feature engineering to select critical predictors, such as cholesterol levels, blood pressure, and age, was crucial in enhancing the model's performance. In contrast, Haque *et al*. (2021) compared Artificial Neural Networks (ANNs) with Logistic Regression for heart disease diagnosis. The ANN outperformed traditional models, achieving an accuracy of 92%, indicating that neural networks' ability to handle non-linear relationships made them better suited for complex medical datasets.

Sivasangari *et al*. (2020) explored Naive Bayes and Decision Trees for liver disease diagnosis, with Naive Bayes achieving an accuracy of 85%. The study highlighted the importance of probabilistic models in handling imbalanced medical datasets, where certain disease categories have significantly fewer samples. Similarly, Rajkumar & Soni (2021) applied Gradient Boosting for coronary heart disease prediction, reaching 91% accuracy. They emphasized the strength of ensemble methods like Gradient Boosting in enhancing classification performance through iterative training. Freeman *et al*. (2019) investigated the use of neural networks in predicting rare pediatric diseases. Their deep learning model achieved over 90% accuracy but required large datasets for effective training, a common limitation of deep learning in healthcare. Naik *et al*. (2021) addressed this by employing ensemble learning techniques such as Random Forest and XGBoost to predict genetic disorders, achieving an accuracy of 97.2%. Ensemble methods provided improved generalization by combining multiple classifiers, reducing the likelihood of overfitting. Li *et al*. (2020) utilized Convolutional Neural Networks (CNNs) for breast cancer detection from histopathology images.

Their model achieved an accuracy of 96.5%, demonstrating that CNNs excel in the tasks of medical imaging due to their ability to automatically extract hierarchical features. Similarly, Ahmed *et al*. (2019) applied K-Nearest Neighbors (KNN) for hypertension prediction, achieving 85% accuracy. Despite its simplicity, KNN was effective in this task, though careful tuning was necessary to prevent performance degradation with large datasets. Zhou *et al*. (2018) implemented Decision Trees to forecast rheumatoid arthritis using patient health records, achieving an accuracy of 87.9%. They stressed the need for high-quality data, noting that incomplete or noisy records significantly impacted the model's performance. In contrast, Miller *et al*. (2020) used Random Forests for Alzheimer's disease prediction, combining genetic and clinical data. Their model achieved 88% accuracy, highlighting the importance of integrating different data sources to improve disease prediction accuracy.

Khan & Zubair (2021) conducted a study on chronic kidney disease (CKD) prediction, applying Decision Trees, Gradient Boosting and Random Forest. Gradient Boosting emerged as the best-performing model with a 94% accuracy, outperforming Random Forest, which achieved 92%. This study reinforced the notion that ensemble methods can significantly enhance prediction accuracy by reducing variance and improving the model's ability to generalize. In their research, Williams *et al*. (2021) utilized Gradient Boosting to forecast the progression of Parkinson's disease, achieving an accuracy of 93%. Their findings emphasized the effectiveness of ensemble models in capturing the nuanced advancement of neurodegenerative diseases, which often entail intricate and interconnected symptoms. Similarly, Cortes *et al*. (2020) investigated the application of Support Vector Machines (SVM) in forecasting asthma exacerbations, attaining an accuracy of 86.7%. SVM proved to be particularly adept at handling feature spaces with high dimensions, a common characteristic of medical datasets containing numerous symptoms and biomarkers. Rathi & Pareek (2016) devised a hybrid machine learning model that combined Decision Trees and Naive Bayes to predict diverse illnesses, including diabetes and heart disease. Their model achieved an overall accuracy of 88%, highlighting the advantages of integrating multiple algorithms to harness their individual strengths. In a seminal study, Littell (1994) underscored the significance of technological innovation in medicine, forecasting the pivotal role of artificial intelligence and machine learning in the future of healthcare diagnostics.

## PROPOSED METHODOLOGY

**Data Acquisition and Exploration**

We obtained an extensive dataset of symptoms and diseases from Kaggle, which included 132 symptom columns and a single target column indicating the diagnosis. After checking for missing values, any gaps were addressed using imputation methods. We conducted exploratory data analysis (EDA) by utilizing bar plots to examine the distribution of disease frequencies and ensure the balance of the data. The overall flowchart of disease prediction is visualized in Figure 1.

**Data Preprocessing**

This stage included the following actions, Label Encoding: Using Python's Label Encoder, categorical variables were converted into numerical labels. In Train-Test split, the dataset was divided into training (70%) and testing (30%), ensuring sufficient data for model assessment. The sample data of disease prediction from the dataset has represented in Figure 2.

**Data Cleaning and Feature Engineering**

Thorough cleaning of the dataset was necessary, involving imputation for missing data and encoding the target variable. Feature engineering comprised the selection of relevant symptoms as features while discarding irrelevant ones, and the use **of Recursive Feature Elimination (RFE)** to rank features by importance. Exploratoring the data by Visualize the dataset to understand the distribution of diseases (prognosis). Utilize tools like Seaborn to create visualizations such as bar plots to depict the frequency of each disease, aiding in understanding the dataset's class distribution. Visualization of the dataset to understand the distribution of diseases has represented in figure 3.

**Model Selection and Training**

In the process of model selection and training, we opted for SVM, Naive Bayes, and Random Forest models based on their performance in similar disease prediction studies. These models underwent training using the training dataset, and we utilized 8-fold cross-validation to optimize hyperparameters. The classifiers implemented were as follows: For SVM, we employed training with a radial basis function (RBF) kernel and fine-tuned regularization parameters suitable for healthcare datasets. Naive Bayes utilized Gaussian NB to effectively handle continuous medical data. In the case of Random Forest, we trained an ensemble consisting of 100 decision trees to minimize variance and enhance prediction accuracy.

**Evaluation Model and Metrics**

For the evaluation of the disease prediction models, we used the benchmark metrics. These metrics facilitated a comprehensive understanding of the models' performance on both training and testing data. In order to enhance prediction accuracy, we amalgamated the predictions from the three classifiers through majority voting. This approach ensured that the overall system could yield accurate results even if one model made an incorrect prediction.

## RESULTS AND DISCUSSION

**Performance of Proposed Model**

The performance of each model was evaluated using cross-validation scores **Support Vector Machine** achieved an average accuracy of **96.2%** and displayed a well-distributed confusion matrix. **Naive Bayes** showed slightly lower performance, with an accuracy of **93.8%,** mainly due to its probabilistic nature and sensitivity to data imbalances. **Random Forest** yielded the best results, achieving **98.1%** accuracy with high precision and recall rates across most disease categories. The ensemble approach improved the overall accuracy to **98.7%,** highlighting the benefit of combining predictions and mitigating the limitations of individual classifiers. This was particularly evident in addressing the challenges experienced by Naive Bayes with certain disease categories.

**Comparison of Models**

In most cases, the Random Forest model performed better than the others because of its capability to manage complex feature interactions. SVM showed strong performance in scenarios with high-dimensional feature spaces, while Naive Bayes offered a suitable probabilistic framework for imbalanced datasets. The accuracy obtained in each algorithm has represented in figure 4 and also represented in column chart in figure 5.

**Analysis of Confusion Matrix**

The confusion matrices for each model displayed where the classifiers made incorrect predictions for diseases. For example, SVM encountered difficulties with diseases that had similar symptoms, whereas Random Forest dealt with these instances more effectively due to its ensemble nature.The visualization of analysis in a Confusion matrices has been represented in the figure 6, figure 7, figure 8.

**4.4: Analysis of Errors**

The main sources of error were associated with the overlapping symptoms of diseases. For example, flu-like symptoms were frequently mistaken for predictions of common cold or allergies, highlighting the need for more detailed features or additional training datasets. Finally, we will be defining a function that takes symptoms separated by commas as input, predicts the disease based on the symptoms by using the trained models, and returns the predictions in a JSON format. Final prediction of all the models has been represented in the figure 9 .

## CONCLUSION

Disease prediction using machine learning is poised to revolutionize healthcare by enabling early detection, personalized treatment, and efficient resource utilization. By analyzing vast and complex datasets, machine learning models can uncover patterns and risk factors that aid in the early diagnosis of diseases, potentially saving lives and reducing treatment costs. These predictive models support personalized medicine, allowing healthcare providers to tailor treatments to individual patient needs, thereby enhancing treatment efficacy and minimizing adverse effects. Despite the significant promise, the implementation of machine learning in healthcare comes with challenges, including ensuring data privacy, mitigating biases, integrating with clinical workflows, and maintaining models over time. Addressing these challenges requires collaboration across the healthcare and data science communities, as well as adherence to ethical standards and regulatory requirement. As technology advances and data availability improves, the accuracy and utility of machine learning models will continue to grow, making them indispensable tools in modern healthcare. Ultimately, the integration of machine learning into healthcare systems promises to improve patient outcomes, streamline clinical processes, and contribute to more sustainable and effective healthcare delivery.

**Future Suggestions**

Improving Data Enhancing Data such as utilizing SMOTE could address imbalances in classes and enhance the overall performance of the model. Integrating Temporal Data: The inclusion of patient history and time-series data could notably enhance the accuracy of the model, especially for chronic illnesses. Utilize tools such as SHAP (Shapley Additive explanations) to provide explanations for model decisions, ensuring transparency for clinicians

It is essential to ensure that patient data protection complies with HIPAA and GDPR. Furthermore, it is important to continuously monitor models for biases in order to prevent unfair treatment of specific patient groups. Utilize tools such as SHAP (Shapley Additive explanations) to provide explanations for model decisions, ensuring transparency for clinicians.

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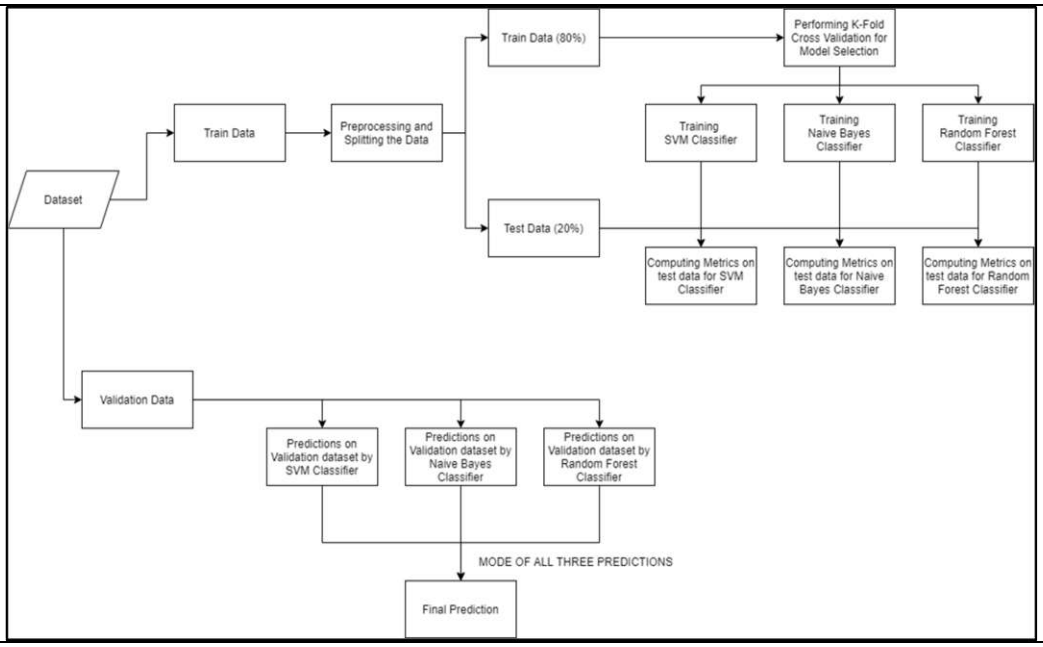


**Fig 1: The flowchart of the proposed methodology.**

```
   itching  skin_rash  nodal_skin_eruptions  continuous_sneezing  shivering  \
0        1          1                     1                    0          0
1        0          1                     1                    0          0
2        1          0                     1                    0          0
3        1          1                     0                    0          0
4        1          1                     0                    0          0

   chills  joint_pain  stomach_pain  acidity  ulcers_on_tongue  ...  \
0       0           0             0        0                 0  ...
1       0           0             0        0                 0  ...
2       0           0             0        0                 0  ...
3       0           0             0        0                 0  ...
4       0           0             0        0                 0  ...

   blackheads  scurring  skin_peeling  silver_like_dusting  \
0           0         0             0                    0
1           0         0             0                    0
2           0         0             0                    0
3           0         0             0                    0
4           0         0             0                    0

   small_dents_in_nails  inflammatory_nails  blister  red_sore_around_nose  \
0                     0                   0        0                     0
1                     0                   0        0                     0
2                     0                   0        0                     0
3                     0                   0        0                     0
4                     0                   0        0                     0

   yellow_crust_ooze         prognosis
0                  0  Fungal infection
1                  0  Fungal infection
2                  0  Fungal infection
3                  0  Fungal infection
4                  0  Fungal infection

[5 rows x 133 columns]
```

**Fig 2: The sample data from dataset**

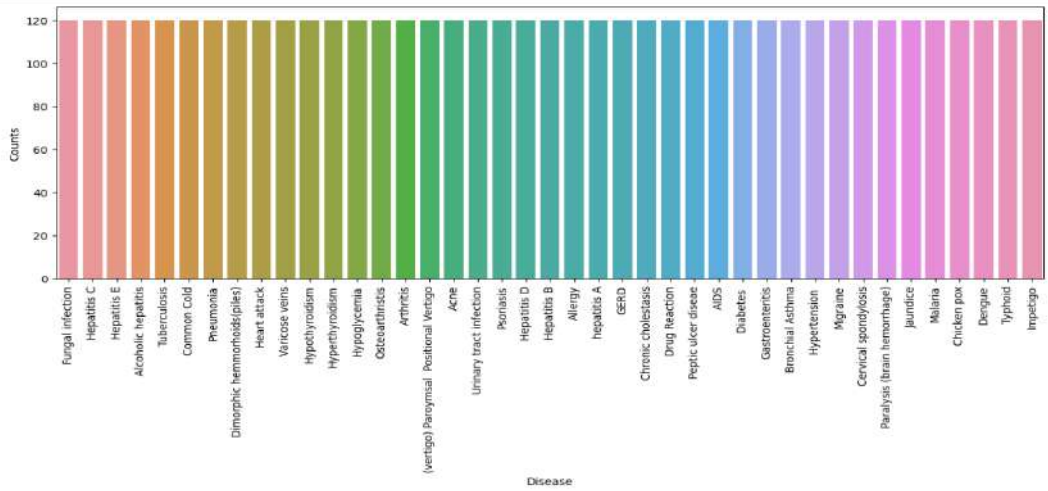


**Fig 3: Visualization of balanced dataset**

| S.NO | PROPOSED ALGORITHMS | ACCURACY |
|---|---|---|
| 1. | Support Vector Machine | 96.2% |
| 2. | Navie bayes classifier | 93.8% |
| 3. | Random forest classifier | 98.1% |

**Fig 4: Accuracy of all the three models .**

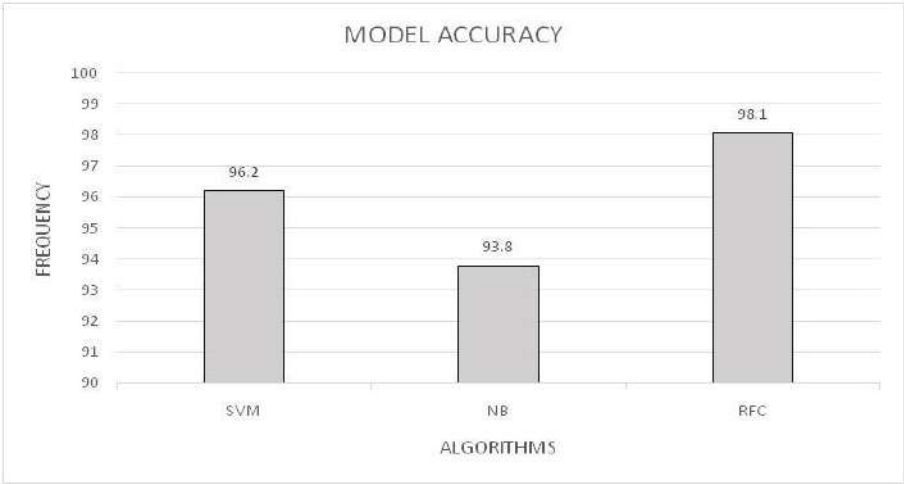


**Fig 5:Visulization of prediction via chart.**

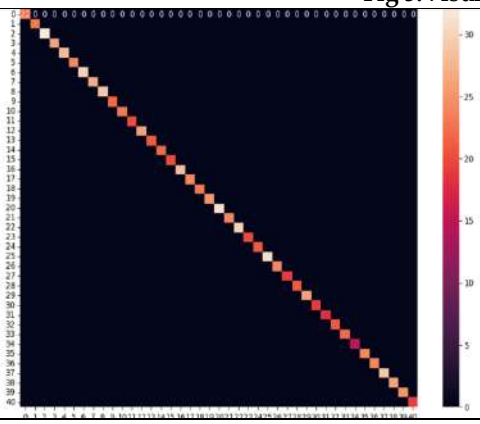

**Fig 6: Support vector machine**

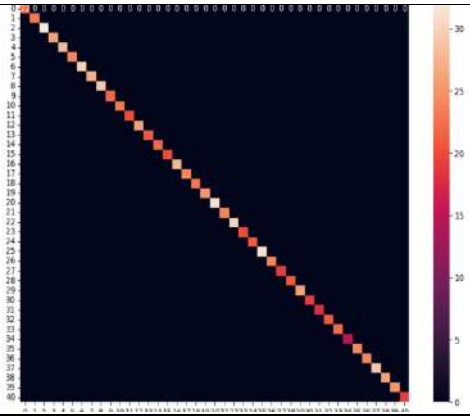

**Fig 7: Navie bayes classifier**

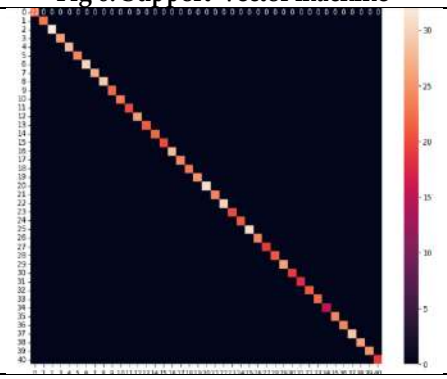

**Fig 8: Random forest classifier**

```
{
    'rf_model_prediction': 'Fungal infection',
    'naive_bayes_prediction': 'Fungal infection',
    'svm_model_prediction': 'Fungal infection',
    'final_prediction': 'Fungal infection'
}
```

**Fig 9: final prediction of all the models .**

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**RESEARCH ARTICLE**

# Audio Feature Extraction and Analysis using LibROSA

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**ABSTRACT**

Audio feature extraction is crucial in various fields including speech recognition, music analysis, emotion detection, and multimedia content retrieval. This paper focuses on developing a robust framework for extracting meaningful audio features using Python and machine learning techniques. By leveraging Python libraries such as LibROSA, Py-Dub, and SciPy. We aim to efficiently extract both low-level features (e.g., amplitude, pitch, tempo) and high-level features (e.g., timbre, harmonic content, and Mel-frequency cepstral coefficients) from audio signals. The proposed paper is designed to preprocess raw audio data through noise reduction, normalization, and segmentation. These applications may include speech-to-text conversion, genre classification, speaker recognition and emotion detection based on voice intonations. The paper's primary goal is to automate the extraction process, enabling accurate analysis of audio data in real-time. This system holds significant potential for use in industries such as music production, entertainment, and healthcare, where audio analysis is essential. The extracted features can also serve as input data for further machine learning models, facilitating tasks like audio classification and sentiment analysis.

**Keywords:** LibROSA, Mel-frequency cepstral coefficient, amplitude, Py-Dub.

## INTRODUCTION

Audio feature extraction is a fundamental approach in the domains of machine learning and audio signal processing. It involves converting unprocessed audio input into meaningful numerical representations that can be applied to a

range of tasks, such as multimedia content retrieval, emotion detection, voice recognition, and music analysis.Strong machine learning algorithms that convert complex audio signals into a set of numerical features improve our capacity to evaluate and understand the underlying aspects of the audio content. These attributes can capture information about the loudness, frequency, timbre, rhythm, and other relevant aspects of the audio stream. This review paper aims to provide a comprehensive overview of audio feature extraction techniques, their applications, and the associated opportunities and challenges. Audio feature extraction is a fundamental method in machine learning and audio signal processing. Doing various tasks, such as voice recognition, music analysis, and multimedia content retrieval, involves collecting raw audio input and converting it into useful numerical representations. By converting complex audio signals into a set of numerical features, powerful machine learning techniques improve our capacity to evaluate and understand the underlying qualities of the audio content. The volume, frequency, timbre, rhythm, and other relevant aspects of the audio signal can all be recorded with these features.

**Audio Signal Processing**

An audio signal analysis, property extraction, behavior prediction, pattern recognition, and correlation between signal and other comparable signals are all common tasks of audio signal processing algorithms. There are three types of audio signals: speech, music, and ambient noise. A tremendous growth in signal analysis and classification has been observed in audio signal processing during the past few decades. It has been demonstrated that a multitude of current problems can be resolved by the amalgamation of contemporary machine learning (ML) algorithms with audio signal processing methodologies. Each machine learning algorithm's performance is determined by the features that are used for testing and training. The purpose of this research is to compile the body of knowledge on audio signal processing, with a particular emphasis on feature extraction methods. The aspects of the temporal, frequency, cepstral, wavelet, and time-frequency domains are covered in detail in this survey. A representation of sound is called an audio signal. Analog and digital are the two primary forms of audio signals. Audio captured with techniques that mimic the original sound waves is referred to as analog. Cassette tapes and vinyl records are two examples. The process of recording digital audio involves sampling the original sound wave at a predetermined frequency, known as the sampling rate. MP3 files and CDs are two instances of digital formats. Real-world applications frequently require conversions between digital and analog waveforms. These conversions are made possible by the ADC (Analog-to-Digital Converter) and DAC (Digital-to-Analog Converter), which are components of audio signal processing.

**Audio Feature Extracting Techniques**

An essential phase in audio signal processing is called "audio feature extraction," which converts unprocessed audio input into a set of numerical features that may be applied to a variety of tasks like audio synthesis, retrieval, and classification. Pitch, timbre, rhythm, loudness, and other crucial aspects of the audio stream are captured by these features. Perceptual linear prediction (PLP)is the method that is most frequently employed for audio feature extraction in various purposes.

**Perceptual Linear Prediction (PLP)**

The perceptual linear predictive (PLP) methodology is a novel method for audio analysis that is introduced and discussed. The equal-loudness curve, the intensity-loudness power law, and the critical-band spectral resolution are three ideas from the psychophysics of hearing that are used in this method to estimate the auditory spectrum. Next, we use an autoregressive all-pole model to mimic the auditory spectrum. Details of the audio spectrum that rely on the speaker can be effectively suppressed using a 5th-order all-pole model. PLP technique exhibits greater auditory consistency when compared to traditional linear predictive (LP) analysis. A low-dimensional representation of speech is produced by the computationally efficient PLP analysis. These properties are found to be useful in speaker-independent automatic-speech recognition. An audio feature extraction method called Perceptual Linear Prediction (PLP) mimics the human auditory system to increase the relevance of derived data, especially for speech processing. It employs equal loudness pre-emphasis to accommodate for frequency sensitivity, a crucial band analysis based on the bark scale and the power law of hearing to translate the signal's intensity into perceived loudness. Following this auditory-based transformation, the features of the vocal tract are captured via standard

linear prediction, yielding PLP coefficients that robustly reflect the signal against noise and speaker fluctuations, making it suitable for applications such as speech recognition.

**Low Level Feature Extraction**

Analysing and computing basic, fundamental properties from audio signals is known as low-level feature extraction. These waveform-derived qualities give crucial information about the audio's structure and substance without requiring sophisticated semantic comprehension. comprehension of the dynamics, tonal qualities, and rhythmic patterns of audio, respectively, depends on a comprehension of the three main aspects of audio that are covered here: amplitude, pitch, and tempo.

**Amplitude**

Capturing the loudness or energy of an audio stream is known as amplitude extraction in machine learning, and it is an essential component of many audio processing tasks. The process starts with the raw audio data being pre-processed, which involves dividing the signal into short frames, usually lasting 20 to 50 milliseconds. Each frame has windowing functions like Hann or Hamming applied to it to minimize edge effects, and techniques like average amplitude or root mean square (RMS) are used to calculate the amplitude for each frame. To comprehend the variation of the amplitude distribution over various frequencies, spectral parameters associated with amplitude, like the spectral flux and spectral centroid, can also be estimated. Upon extraction, the amplitude-related features are fed into several machine-learning models. While unsupervised learning techniques like clustering with k-means or Gaussian Mixture Modelsgroup signals with similar amplitude patterns in situations where labels are unavailable, supervised learning models like Support Vector Machines and Neural Networks can be trained on labelled datasets to classify audio signals based on their amplitude characteristics. More complex deep learning models, including Recurrent Neural Networks and Convolutional Neural Networks, can be used directly on spectrograms or raw waveforms to automatically identify patterns related to amplitude. These models frequently perform better than conventional techniques in tasks like audio event detection, voice recognition, and music genre classification especially when trained on big datasets.

**Pitch**

Machine learning-based pitch extraction in audio aims to pinpoint a sound's basic frequency, which is important for applications like voice recognition and music analysis. Pitch extraction has historically relied on methods like autocorrelation or cepstral analysis, but machine learning provides more sophisticated options, particularly in complex or noisy contexts. This method involves first preprocessing the audio input to capture the harmonic structure through framing and the application of transformations such as the Short-Time Fourier Transform. Next, pitch-related characteristics that are taken out of the signal are used to train machine learning models. Deep learning models, especially Long Short-Term Memory networks, are very good at learning pitch temporal correlations. Pitch extraction driven by machine learning finds use in voice analysis, audio classification, melody identification, and music transcription. It works exceptionally well in loud areas where other approaches are less effective. Machine learning has greatly increased the accuracy and dependability of pitch extraction in contemporary audio processing, despite obstacles like the requirement for huge datasets and computational power.

**Tempo**

Finding the tempo, or beats per minute (BPM), of a musical composition or rhythmic audio clip is known as "tempo extraction in audio using machine learning." Tempo is a crucial component in beat tracking, rhythm identification, and music analysis. Conventional techniques like beat detection algorithms or autocorrelation are frequently employed. But more resilient and adaptive methods have been provided by machine learning, particularly for managing loud and complicated audio data. The audio input is first pre-processed in machine learning-based tempo extraction by splitting it into frames and using transformations like the Short-Time Fourier Transform to record the frequency and amplitude variations across time. The audio is processed to extract features, or temporal patterns, which are rhythmic beats. The key to determining pace is to observe the periodicity of beats, which is reflected in

these patterns. Using labeled datasets with known tempo, machine learning models, like Support Vector Machines (SVM) or Decision Trees, can be taught to recognize rhythmic patterns that correspond with particular BPM values.

**High Level Feature Extraction**

**Mel-Frequency Cepstral Coefficient**

Speech signals, random signals, and independent carrying signals are all naturally occurring and time-dependent. Since speech signals can be classified based on these features, it is vital to extract the speech signal's properties or features for analysis of speech audio signals. The suggested method usesMATLAB programming to implement the Mel-frequency cepstral coefficients (MFCC) technique for feature extraction analysis of spoken word wav files. Due to its robustness, effectiveness, and ease of implementation, the MFCC feature extraction technique is frequently employed in voice recognition.

Any machine learning method's performance is greatly impacted by the extraction and representation of features. Mel Frequency Cepstral Coefficientis a frequently used model in many domains to simulate the characteristics of an audio signal. The purpose of this work is to review the applications of MFCC as well as various problems with MFCC calculation and how they affect model performance. These concerns include using MFCC for non-acoustic signals, using time series instead of a global representation of the MFCC, using the standard form of the MFCC computation rather than changing its parameters and providing deep learning techniques versus traditional machine learning techniques.

**Harmonic Content**

Understanding a sound signal's harmonic components is essential to comprehending its timbral properties and overall quality. This is the emphasis of machine learning-based harmonic content extraction in audio. Harmonics which are the additional frequencies at which a sound vibrates in addition to its fundamental frequency, are important in determining the tonal characteristics of voices, musical instruments, and other audio sources. Preprocessing the audio signal is the first step in machine learning-based harmonic content extraction. Typically, methods like harmonic product spectrum are used to split the signal into its frequency components. These techniques aid in separating harmonic content from non-harmonic elements and noise. These features are analysed using machine learning models, which include supervised and unsupervised methods. Labelled datasets can be used to train supervised models, like Support Vector Machines and Neural Networks, to predict or classify harmonic qualities using recognized patterns. By identifying patterns in the frequency domain and differentiating between harmonic and non-harmonic components, these models acquire the ability to identify harmonic content. Convolutional Neural Networks, one type of deep learning approach, are very good at learning intricate patterns from spectrograms, which allow harmonic structures to be seen and examined in great detail. Applications for machine learning-driven harmonic content extraction include pitch estimation, audio source separation, and the recognition of musical instruments. It makes it feasible to analyse audio signals with greater precision and detail, enabling the identification of particular harmonic elements that add to the sound's character and quality. The accuracy and efficiency of harmonic content extraction have been considerably improved by machine learning approaches, opening the door for more sophisticated audio processing and analysis tools, notwithstanding the difficulties associated with requiring huge and diverse datasets and substantial computer resources.

**Python Libraries and Tools Used**

**LibROSA**

Working with audio files is made easier with LibROSA high-level interface and extensive feature set. Preprocessing audio data, feature extraction, visualization, analysis, and even more complex methods like audio source separation and music genre categorization may all be done with it. NumPy and SciPy are core libraries for Python scientific computing, upon which LibROSA is constructed. It offers a collection of modules and submodules for managing various facets of audio files. The essential features of LibROSA, such as time stretching, resampling, and audio file loading, are included in this module. The Mel-spectrogram, spectral contrast, chroma characteristics, zero crossing

rate, and temporal centroid are only a few of the audio features that are extracted by this module. This paper has utilities for visualizing spectrograms, audio waveforms, and other related visualizations, as its name suggests. This module provides audio processing and manipulation features such audio segmentation, noise reduction, and time and pitch shifting. Advanced approaches like audio source separation, speech emotion identification, and music genre categorization are covered in this module. It may be simply installed and then imported into your Python environment so you can begin working with audio files.

Preprocessing the audio data is crucial to ensuring its quality and compatibility with the intended analysis methodologies before beginning any audio analysis. It offers a number of preparation operations for audio data, such as scaling, time stretching, resampling, audio normalization, and handling missing data. As it assists in capturing the pertinent properties of the audio stream, feature extraction is an essential stage in audio analysis. LibROSA provides a number of functions, including zero crossing rate, temporal centroid, chroma characteristics, spectral contrast and mel spectrogram, for the purpose of extracting audio features. Speech recognition, sound event detection, and music genre classification can all benefit from these features.

**Py-Dub**

Py-Dub is a robust Python package that is frequently used for preprocessing tasks including feature extraction and audio file editing. Its ability to load, trim, and convert audio files into many formats (such as MP3, WAV, and OGG) makes it an essential tool in the early stages of an audio analysis pipeline, even if it is not primarily designed for extracting audio features. In order to guarantee that the audio data is in a consistent and comprehensible format, Py-Dub may also carry out essential preprocessing operations like resampling audio to a specific sample rate, normalizing volume levels, eliminating silence, and converting stereo to mono. Before transferring the audio data to more sophisticated feature extraction software, these processes are frequently necessary. Mel-frequency cepstral coefficients (MFCCs), spectral contrast, chroma characteristics, and zero-crossing rate are just a few of the audio features that can be extracted using Py-Dub preprocessing and libraries like LibROSA or python speech features. For tasks like voice recognition, acoustic scene analysis, and music genre categorization, these aspects are crucial. Workflow flexibility and efficiency are achieved by combining Py-Dub's preprocessing capabilities with the sophisticated feature extraction methods of specialist libraries. The accuracy and integrity of the data are maintained while even the most complicated audio analysis tasks may be completed with ease thanks to this connection.

**SciPy**

Utilized extensively in scientific and numerical computing, the SciPy library provides useful capabilities for audio signal processing—a prerequisite for audio feature extraction. SciPy is an essential tool for preprocessing and basic signal processing tasks, even though it is not a library specifically designed for audio analysis. To begin the process of extracting significant features from audio data, the SciPy.Signal submodule offers a number of functions for filtering, transforming, and analysing audio signals. SciPy can be used, for instance, to execute Fourier Transforms, which are required to transfer audio signals from the time domain to the frequency domain in order to analyse the spectrum content of audio data. When extracting features like the bandwidth, chroma features, and spectral centroid, this is frequently a crucial step. SciPy facilitates the creation of spectrograms through the utilization of the Short-Time Fourier Transform, allowing for both quantitative and visual examination of the temporal variations in an audio signal's frequency content. Additionally, SciPy enables the application of filters, including as band-pass, low-pass, and high-pass filters, which are frequently used as a preprocessing step before extracting audio characteristics. For tasks like speech recognition and music analysis, temporal parameters like onset, attack time, and peak intensity must be calculated. SciPy's ability to extract the amplitude envelope and detect signal peaks is very helpful in these calculations. A strong feature extraction pipeline can be constructed by integrating SciPy with other specialized audio libraries like lib ROSA. Signal processing, which offers a strong basis for more complex audio feature extraction, is one of SciPy's strongest points. Higher-level features like Mel-frequency cepstral coefficients, zero-crossing rate, and spectral contrast, for instance, can be extracted from the cleaned signal by passing it via libraries like LibROSA after filtering or running an FFT with SciPy. This integration makes sure that audio data is effectively handled and ready for more in-depth examination.

**Challenges and Future Directions**

Despite being widely used in fields including music analysis, speech recognition, and environmental sound classification, audio feature extraction still has a number of issues that prevent it from reaching its full potential. Managing noisy or real-world data is one of the main difficulties. Uncontrolled situations frequently introduce background noise, reverberation, or overlapping sound sources into audio signals, which can skew the features that are extracted and impair the efficiency of tasks that follow. While modern methods such as signal augmentation or noise reduction can help reduce these problems, they are not always successful in keeping the important aspects intact. A further difficulty is the unpredictability of audio data, which can greatly affect the consistency of features that are retrieved. Examples of these variances include differences in speaker accent, pitch, and recording settings. Future research efforts are becoming more focused on creating feature extraction techniques that are more resilient and adaptable so they can deal with noisy and varied data sets more skilfully. Using deep learning methods in conjunction with feature extraction is one such path. Convolutional neural networks and recurrent neural networksin particular are deep learning models that, despite their high CPU resource requirements and massive dataset requirements, have demonstrated promise in automatically extracting useful features from unprocessed audio without the need for human intervention. Further investigation should focus on increasing computational efficiency, particularly for real-time applications in edge computing and Internet of Things contexts. There is potential for improvement in domain-specific feature extraction, where features are customized for certain audio applications like sound event identification or emotion recognition.

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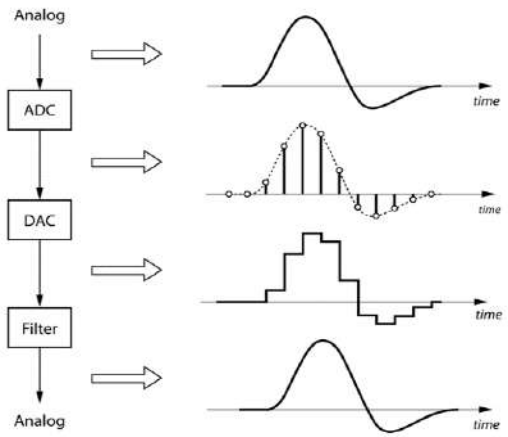


Fig 1 : Audio signal processing

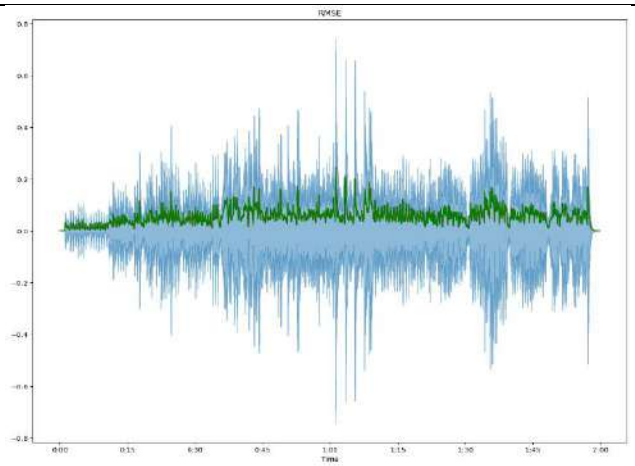


Fig 2 : Visualization of amplitude in audio

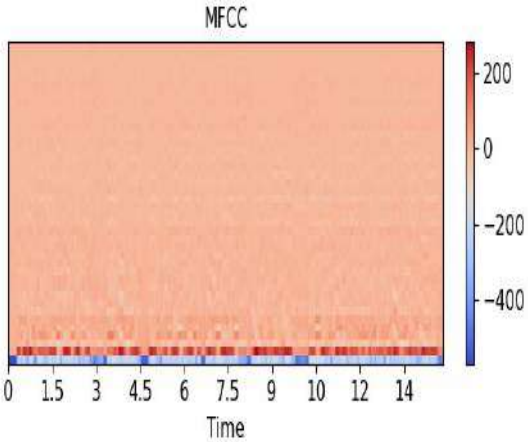


**Fig 3 :** Visualization of MFCC using LibROSA

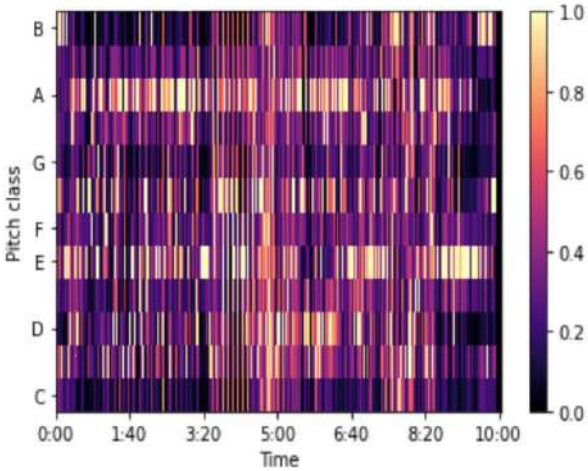


**Fig 4 :** Visualization of audio using LibROSA

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**RESEARCH ARTICLE**

# Feature Extraction using Image mining Techniques

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**ABSTRACT**

The image mining technique deals with the mining of hidden facts and image with data association or other patterns not unambiguously stored in the images. Enhancements in image acquisition and storage mechanism have led to fabulous escalation in radically large and detailed image databases. These images, if analyzed, can expose valuable information to the human users. Image mining is more than just a conservatory of data mining to image realm. In this paper, we will observe the research issues in image mining, contemporary developments in image mining, predominantly, up to date techniques and systems. Our study is to mine unseen information from Images – to mine patterns and obtain knowledge from large gathering of images, agreements mainly with recognition and extraction of unique features for a particular domain.

**Keywords:** technique, predominantly, knowledge, study.

## INTRODUCTION

The World Wide Web is regarded as the prevalent large-scale image repository. An extremely outsized amount of image data such as satellite images, medical images, and digital photographs are spawned every day. These images, if analyzed, can divulge constructive information to the human users. Unfortunately, there is a lack of effectual tools for searching and finding useful patterns from these images. Image mining systems that can involuntarily extract semantically important information (knowledge) from image data are gradually more in demand [1]. The fundamental challenge in image mining is to determine how low-level, pixel representation contained in a raw image or image sequence can be efficiently and effectively processed to identify high-level spatial objects and relationships. In other words, image mining deals with the extraction of implicit knowledge, image data relationship, or other patterns not explicitly stored in the image databases. It is an interdisciplinary endeavor that essentially draws upon expertise in computer vision, image processing, image retrieval, data mining, machine learning, database, and

artificial intelligence [2]. While some of the individual fields in themselves may be quite matured, image mining, to date, is just a growing research focus and is still at an experimental stage. The main obstacle to rapid progress in image mining research is the lack of understanding of the research issues involved in image mining. Many researchers have the wrong impression that image mining is just a simple extension of data mining applications; while others view image mining as another name for pattern recognition. In this paper, we attempt to identify the unique research issues in image mining. This will be followed by a review of what are currently happening in the field of image mining, particularly, image mining frameworks, state-of-the-art techniques and systems. We will also identify possible research directions to bring image mining research to a new height [3].

**Image mining techniques**

Besides investigating suitable frameworks for image mining, early image miners have attempted to use existing techniques to mine for image information. The techniques frequently used include object recognition, image indexing and retrieval, image classification and clustering, association rules mining, and neural network. Object recognition has been an active research focus in field of image processing. Using object models that are known a priori, an object recognition system finds objects in the real world from an image. This is one of the major tasks in the domain of image mining. Automatic machine learning and meaningful information extraction can only be realized when some objects have been identified and recognized by the machine. The object recognition problem can be referred to as a supervised labeling problem based on models of known objects. Specifically,given a target image containing one or more interesting objects and a set of labels corresponding to a set of models known to the system, what object recognition does is to assign correct labels to regions, or a set of regions, in the image. Models of known objects are usually provided by human input a priori [3].

Traditional data mining techniques have been developed primarily for planned datatypes. The image datatype does not belong to this planned category, apt for elucidation by a machine, and hence the mining of image data is a exigent problem. Content of an image is visual in nature and the analysis of the information conveyed by an image is mainly subjective, based on the human visual system.

The image retrieval systems can be broadly categorized into two categories based on the type of searches, using either description of an image or its visual content. In the first category, the images are described based on user-defined texts [4, 5]. The images are indexed and retrieved based on these rudimentary descriptions, such as their size, type, date and time of capture, identity of owner, keywords, or some text description of the image. As a result, this is often called description based or text-based image retrieval process. This second category of similarity based image retrieval process is called Content Based Image Retrieval (CBIR) [6]-[10]. In CBIR systems, the images are searched and retrieved based on the visual content of the images. Based on these visual contents, desirable images features can be extracted and used as index or basis of search. Content-based image retrieval is highly desirable and has increasingly become a growing area of study towards the successful development of image mining techniques.

**Content-Based Image Retrieval**

There are, in general, three fundamental modules in a content-based image retrieval system. These are

1. Visual content or feature extraction,
2. Multidimensional indexing, and
3. Retrieval.

The images in an image database are indexed-based on extracted inherent visual contents (or features) such as color, texture, pattern, image topology, shape of objects and their layouts and locations within the image, etc. An image can be represented by a multidimensional vector of the extracted features from the image. The feature vector actually acts as the signature of the image. This feature vector can be assumed to be associated to a point in the multidimensional space. The query image can be analyzed to extract the visual features and can be compared to find matches with the indices of the images stored in the database.

**Color features**

Color is one of the most widely used visual features in content based image retrieval [11]-[14]. While we can perceive only a limited number of gray levels, our eyes are able to distinguish thousands of colors and a computer can represent even millions of distinguishable colors in practice. Color has been successfully applied to retrieve images, because it has very strong correlations with the underlying objects in an image. Moreover, color feature is robust to background complications, scaling, orientation, perspective, and size of an image. A color pixel in a digital image is represented by three color channels (usually Red, Green and Blue). It is well known that any color can be produced by mixing these three primary colors.

**Texture features**

Texture is a very interesting image feature that has been used for characterization of images, with application in content-based image retrieval. There is no single formal definition of texture in the literature. However, a major characteristic of texture is the repetition of a pattern or patterns over a region in an image. The elements of patterns are sometimes called textons. The size, shape, color, and orientation of the textons can vary over the region. The difference between two textures can be in the degree of variation of the textons. It can also be due to spatial statistical distribution of the textons in the image. Texture is an innate property of virtually all surfaces, such as bricks, fabrics, woods, papers, carpets, clouds, trees, lands, skin, etc. It contains important information regarding underlying structural arrangement of the surfaces in an image. When a small area in an image has wide variation of discrete tonal features, the dominant property of that area is texture. On the other hand, the gray tone is a dominant property when a small area in the image has very small variation of discrete tonal features. Texture analysis has been an active area of research in pattern recognition since the 1970s [15, 16]. Several texture features such as entropy, energy, contrast, and homogeneity, can be extracted from the co-occurrence matrix of gray levels of an image [17].

**Shape features**

Shape is another image feature applied in CBIR. Shape can roughly be defined as the description of an object minus its position, orientation and size. Therefore, shape features should be invariant to translation, rotation, and scale, for an effective CBIR, when the arrangement of the objects in the image is not known in advance. To use shape as an image feature, it is essential to segment the image to detect object or region boundaries; and this is a challenge. Techniques for shape characterization can be divided into two categories. The first category is boundary-based, using the outer contour of the shape of an object. The second category is region-based, using the whole shape region of the object. The most prominent representatives of these two categories are Fourier Descriptors [18] and Moment Invariants [19]. The main idea behind the Fourier Descriptors is to use the Fourier-transformed boundaries of the objects as the shape features, whereas the idea behind Moment Invariants is to use region-based geometric moments that are invariant to translation and rotation. Hu identified seven normalized central moments as shape features, which are also scale invariant.

**Topology**

A digital image can be represented by one or more topological properties [20], which typically represent the geometric shape of an image. The interesting characteristic of topological properties is that when changes are made to the image itself, such as stretching, deformation, rotation, scaling, translation, or other rubber-sheet transformations, these properties of the image do not change. As a result, topological properties can be quite useful in characterization of images and can be used as a signature of an image content to use in content based image retrieval. One topological property of a digital image is known as Euler number [21].The Euler number is usually computed in a binary image. However, it can be extended to characterize gray-tone images as well by defining a vector of Euler numbers of the binary planes of the gray-tone image.

**Multidimensional indexing**

Multidimensional indexing is an important component of content-based image retrieval. Development of indexing techniques has been an active area in database management, computational geometry, and pattern recognition. However, the notion of indexing has subtle difference in different communities. The notion of indexing in

multimedia data mining and content-based image retrieval is different than its notion in the traditional database management systems. In traditional database management system (particularly for relational database), the indexing refers to the access structure of the database files in terms organization of the records. Indexes are specified based on one or more attributes of the records in order to process queries based on those attributes.

## RESULTS OF A SIMPLE CBIR SYSTEM

Sana et al. proposed a simple image matching scheme for an experimental content-based image retrieval system that uses moment, shape, and texture features extracted from the images [21]. The shape features have been extracted using moment computation (feature set A) of the regions, combined with circularity, aspect ratio, concavity, and symmetricity metrics (feature set B). The texture of the images have been generated using the energy,

- Moment invariants only.
- Circularity, symmetricity, aspect ratio and concavity.
- Energy, entropy, contrast and homogeneity.

entropy, contrast, and homogeneity measure as image features (set C). We present here results of this system using around 290 samples taken from an image database containing several classes of images, including animals, cars, flowers, etc. Distance of the query image from the database images have been computed simply as $\Sigma|fi - fi'|$ where fi and fi′ are the values of the ith feature of the database image and query image respectively. The top ten closest images have been taken as the query result, excluding the query image itself if it is present in the database.

Tables 1 and 2 provide the results, where each database image is used as a query image to find the top ten from the database. From Table.1, we observe that the overall performance can be improved by mixing the different feature sets for image query.

**Research issues in image mining**

Image mining deals with the extraction of image patterns from a large collection of images. Clearly, image mining is different from low-level computer vision and image processing techniques because the focus of image mining is in extraction of patterns from *large* collection of images, whereas the focus of computer vision and image processing techniques is in understanding and/or extracting specific features from a *single* image. While there seems to be some overlaps between image mining and content-based retrieval (both are dealing with large collection of images), image mining goes beyond the problem of retrieving relevant images. In image mining, the goal is the discovery of image patterns that are significant in a given collection of images. Perhaps, the most common misconception of image mining is that image mining is nothing more than just applying existing data mining algorithms on images. This is certainly not true because there are important differences between relational databases versus image databases [3].

In addition to the need for new discovery algorithms for mining patterns from image data, a number of other related research issues also need to be resolved. For instance, for the discovered image pattern to be meaningful, they must be presented visually to the users. This translates to following issues:

(a) Image pattern representation. How can we represent the image pattern such that the contextual information, spatial information, and important image characteristics are retained in the representation scheme?

(b) Image features selection. Which are the important images features to be used in the mining process so that the discovered patterns are meaningful visually?

(c) Image pattern visualization. How to present the mined patterns to the user in a visually-rich environment? [3].

# CONCLUSION

In summary, image mining is a promising field for research. This paper presents a survey on various image mining techniques that was proposed earlier by researchers for the better development in the field of feature extraction in an image. The purpose of the mining is to produce all considerable patterns without prior knowledge of the patterns. Important information can be hidden in images, conversely, few research talk about data mining on them. Image segmentation is the primary phase in image mining. In this paper, we have highlighted the need for image mining in view of the rapidly growing amounts of image data. We have pointed out the unique characteristics of image databases that brought a whole new set of challenging and interesting research issues to be resolved.

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**Table .1 CBIR match performance (%)**

| Image group | *A* | *B* | *A+B* | *A+B+C* |
|---|---|---|---|---|
| AEROPLANE | 71.3 | 69.68 | 73.39 | 73.23 |
| CAR | 78.90 | 74.11 | 78.30 | 82.56 |
| FLOWER | 51.22 | 38.64 | 43.64 | 43.48 |
| ANIMAL | 25.44 | 48.45 | 48.89 | 49.56 |
| Overall | 62.70 | 38.90 | 64.80 | 66.55 |

**Table .2 CBIR weighted match performance (%)**

| Image group | All features (No weight) | All features (With weight) |
|---|---|---|
| AEROPLANE | 73.23 | 69.84 |
| CAR | 82.56 | 86.75 |
| FLOWER | 43.48 | 77.03 |
| ANIMAL | 49.56 | 72.44 |
| Overall | 66.55 | 77.79 |

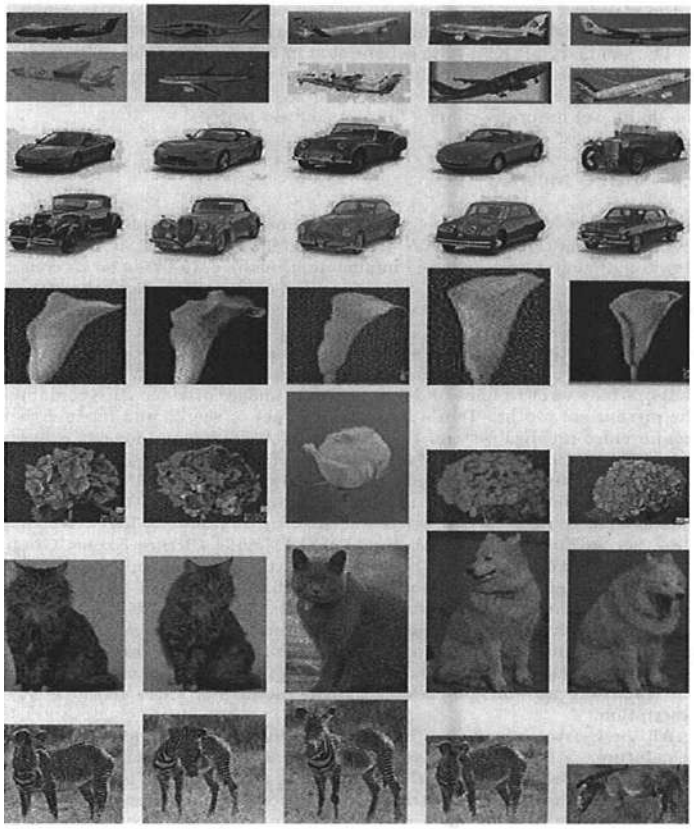

**Fig.. 1 Results of image matching in the CBIR system**

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**RESEARCH ARTICLE**

# Progress in Communication: The Emerging Technologies

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**ABSTRACT**

The evolution of communication technologies has significantly transformed how individuals and organizations interact, disseminate information, and conduct business. This paper explores the advancements in communication technologies over the past few decades, focusing on key innovations such as the internet, mobile communication, and social media. It analyzes their impact on society, including implications for interpersonal relationships, business practices, and global connectivity. By employing a mixed-methods approach that combines a comprehensive literature review with quantitative and qualitative data collection, the research reveals major trends, challenges, and future directions in communication technologies. The findings highlight the importance of adapting to these advancements to enhance communication effectiveness and ensure equitable access to technology.

**Keywords:** communication technologies, internet, mobile communication, social media, global connectivity, information dissemination

## INTRODUCTION

**Preface**

Communication technologies have experienced rapid-fire advancements over the last many decades, unnaturally altering the ways in which individualities and associations connect, partake information, and engage with one another. From the invention of the telegraph to the rise of the internet and mobile communication, these technologies have eased unknown situations of connectivity and information exchange. This paper seeks to explore the progress in communication technologies, examining their elaboration, impact on society, and counteraccusations for the future.

**Purpose of the Study**

The primary purpose of this research is to investigate the significant advancements in communication technologies and their effects on interpersonal relationships, business practices, and global connectivity. The study aims to:

1. Identify key milestones in the development of communication technologies.
2. Analyze the impact of these technologies on social interaction and information dissemination.
3. Explore the challenges associated with the adoption of new communication technologies.
4. Assess future trends and directions in the field of communication technologies.

**Research Questions**

1. What are the major milestones in the evolution of communication technologies?
2. How have advancements in communication technologies affected interpersonal relationships and business practices?
3. What challenges are associated with the adoption of new communication technologies?
4. What are the anticipated future trends in communication technologies?

## LITERATURE REVIEW

The field of communication technologies encompasses a wide range of innovations that have transformed human interaction. Key developments include the internet, mobile communication, and social media, each contributing to the rapid evolution of communication practices.

**The Internet**

The internet has revolutionized communication by enabling instantaneous access to information and facilitating global connectivity. According to Castells (2000), the internet has reshaped social interactions, allowing individuals to connect across geographical boundaries and participate in online communities. The advent of email, instant messaging, and video conferencing has further enhanced communication efficiency, leading to increased collaboration and knowledge sharing.

**Mobile Communication**

Mobile communication has further accelerated the pace of communication, enabling users to stay connected regardless of their location. The proliferation of smartphones has made it possible to access information, communicate, and conduct business on-the-go. According to a report by the Pew Research Center (2019), over 81% of Americans own a smartphone, highlighting the pervasive nature of mobile communication.

**Social Media**

Social media platforms have emerged as significant tools for communication, allowing users to share content, engage with others, and participate in public discourse. Platforms such as Facebook, Twitter, and Instagram have transformed how individuals interact and share information. Research by Kaplan and Haenlein (2010) suggests that social media facilitates both personal and professional networking, fostering connections that were previously difficult to establish.

**Impacts on Society**

The advancements in communication technologies have profound implications for society. They have altered interpersonal relationships, transformed business practices, and influenced cultural norms. However, the rapid pace of technological change also presents challenges, such as information overload, privacy concerns, and the digital divide (Gleason, 2020).

## METHODOLOGY

This research employs a mixed-methods approach to examine the progress in communication technologies. The methodology includes the following components:

**Literature Review**

A comprehensive review of existing literature on communication technologies was conducted to establish a theoretical framework for the study. This review included academic articles, industry reports, and case studies to identify key trends and developments in the field.

**Surveys**

Surveys were administered to a diverse group of individuals, including students, professionals, and educators, to gather data on their experiences and perceptions regarding communication technologies. A total of 300 respondents participated in the survey, providing insights into the impact of technology on their communication practices.

**Case Studies**

In-depth case studies of organizations that have successfully integrated advanced communication technologies into their operations were conducted. These case studies explored how these organizations adapted to technological changes and the benefits they experienced as a result.

**Major Findings**

The research revealed several significant findings regarding the progress in communication technologies and their implications for society.

**Key Milestones in Communication Technologies**

The study identified several key milestones in the evolution of communication technologies:

- The invention of the telegraph (1837), which enabled long-distance communication.
- The development of the telephone (1876), allowing real-time voice communication.
- The rise of the internet (1990s), facilitating global connectivity and information exchange.
- The proliferation of mobile devices (2000s), enhancing communication accessibility.
- The emergence of social media platforms (2000s), transforming social interaction and information sharing.

**Impact on Interpersonal Relationships**

The advancement of communication technologies has significantly influenced interpersonal relationships. The survey results indicated that 75% of respondents felt that technology enhanced their ability to connect with others, while 60% reported that it improved their communication efficiency. However, concerns about the quality of relationships were noted, with 45% expressing that technology sometimes hinders face-to-face interactions.

**Transformation of Business Practices**

Communication technologies have transformed business practices, facilitating remote work, collaboration, and information sharing. The case studies highlighted how organizations that adopted advanced communication tools experienced increased productivity, improved employee engagement, and enhanced customer interactions. Additionally, the survey revealed that 80% of professionals believed that technology had improved their ability to collaborate with colleagues.

**Challenges in Adoption**

Despite the benefits, several challenges were associated with the adoption of new communication technologies. The survey findings indicated that 55% of respondents experienced difficulties in navigating complex technologies, while 50% expressed concerns about data privacy and security. Furthermore, the digital divide remains a significant issue, as individuals without access to technology are at a disadvantage in terms of communication and information access.

## DISCUSSION

The findings of this research underscore the transformative impact of communication technologies on society, particularly in terms of interpersonal relationships and business practices. While advancements have enhanced connectivity and efficiency, challenges remain that must be addressed to ensure equitable access and effective communication.

**Implications for Education**

The rapid evolution of communication technologies necessitates a reevaluation of educational practices. Institutions must integrate technology into curricula to prepare students for the demands of a technology-driven world. This includes fostering digital literacy, critical thinking, and effective communication skills.

**Future Trends in Communication Technologies**

As communication technologies continue to evolve, several trends are anticipated, including the rise of artificial intelligence in communication, the increasing importance of data privacy, and the growth of virtual and augmented reality as communication tools. These advancements will likely further shape the landscape of communication, necessitating ongoing adaptation and learning.

## CONCLUSION

The progress in communication technologies has unnaturally converted how individualities and associations interact, partake information, and conduct business. While these advancements offer multitudinous benefits, they also present challenges that must be addressed to insure indifferent access and effective communication. As society continues to navigate these changes, it's pivotal to foster a culture of rigidity, lifelong literacy, and ethical considerations in the use of communication technologies. unborn exploration should concentrate on exploring the long- term impacts of communication technologies on society, as well as relating strategies for mitigating challenges associated with their relinquishment. By embracing the openings presented by communication technologies, we can enhance connectivity, collaboration, and understanding in an decreasingly connected world.

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**RESEARCH ARTICLE**

# Prediction on Cardiovascular Disease Using Deep Learning Algorithm

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## ABSTRACT

Cardiovascular diseases have accounted for the largest number of deaths globally. An effective policy for treatment and management begins with very early diagnosis and correct diagnosis. Deep learning algorithms are now the most powerful tools that would analyze several data sources, such as EHRs, imaging data, including echocardiography, and biosignals, such as ECGs, to develop the detection of cardiovascular diseases. This review paper covers the recent applications in deep learning for the detection of CVD, promising techniques, challenges, and future directions. The merits and limitations of the various deep learning architectures, especially CNNs and RNNs, will be discussed in analyzing the heterogeneity of data modalities. A discussion of key challenges, including availability and quality of data, model interpretability, and its integration into clinical workflows, is outlined. Besides the factors already discussed, areas in for future studies are the enhancement of data quality and availability, development of interpretable models that would help to build more trust into deep learning, and ways for deep learning to be applied beyond the standard risk prediction use cases such as personalized medicine or real-time monitoring. More generally, this work has the potential to revitalize CVD detection and improve health outcomes related to cardiovascular diseases.

**Keywords:** Deep learning, Convolutional neural networks, deep neural networks, recurrent neural network.

# INTRODUCTION

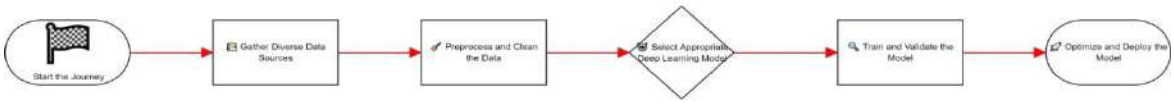
CVDs continue to lead to most deaths, and this is still a grave public health problem at all levels. is a broad term encompassing conditions that affect the heart and blood vessels. It's a leading cause of death worldwide, claiming millions of lives each year. Early detection coupled with proper diagnosis ensures the effective intervention and management of CVDs. In the recent past, deep learning algorithms have emerged as powerful tools in order to enhance the analysis of medical images, improve diagnostic accuracy, and efficiency. Here, we attempt to review the current scenario in which deep learning applications utilize detection, diagnosis, and prognosis in cardiovascular diseases. Based on recent research and developments in this regard, we attempt to grasp trends, challenges, and opportunities for leading deep learning algorithms toward an improvement in cardiac healthcare. Early detection coupled with intervention is crucial for altering patient outcomes. Deep learning algorithms have surfaced as a possibly powerful tool for analyzing medical data and will be helpful in the detection of CVD. This survey explores the current landscape for applications of deep learning in this domain.

**Data Sources for Deep Learning in CVD Detection:**
It is known that deep learning approaches can be aided by various accessible datasets in their cardiovascular disease detection task. The well-known Heart Disease Dataset available at the UCI Repository is also based on the clinical features involved in heart diseases diagnosis. Available at PhysioNet is a wide range of medical datasets, which also include electrocardiograms and medical history related to heart diseases. Besides, many other datasets, for example the one called "Heart Disease UCI", can be found on Kaggle and contain clinical and patient's data. As for clinical records in more detail, the MIMIC-III databases provides a great amount of intensive care records, while NCD Risk Factor Collaboration provides data, this time at the population level, on the risk factors of cardiovascular diseases. The Cleveland Clinic Foundation Heart Disease Dataset is also commonly cited in the CVD researches. Last but not least, health data and its related information from the websites of US government can also be sourced from the HealthData.gov, which may be inclusive of cardiovascular data. These sources also provide a variety of scholarly and practical endeavors in terms of the use of deep learning techniques in CVD detection.
**Electronic Health Records (EHRs):** EHRs contain a wealth of patient information, including demographics, medical history, lab test results, and medication use. Deep learning models can analyze these data points to identify patterns associated with CVD risk.
**Imaging Data:** Techniques like echocardiography and coronary angiography provide visual representations of the heart structure and function. Convolutional Neural Networks (CNNs) excel at extracting features from these images to detect abnormalities indicative of CVD.
**Biosignals:** Electrocardiograms (ECGs) and blood pressure readings capture electrical activity and hemodynamic changes in the heart. Recurrent Neural Networks (RNNs) are adept at learning patterns from sequential biosignal data to predict CVD events.

**Deep Learning Architectures for CVD Detection**
Deep architectures for learning are highly powerful resources for the detection of CVD with a range of data modalities while CNNs prove to be superior in using medical imaging data towards acquiring and classification of structural abnormalities from echocardiograms and cardiac MRI scans. This is recurrent Neural Networks which include Long Short-Term Memory, very suited to sequential data, thus ideal for the analysis of time-series data, such as ECG signals, where the given algorithm can capture temporal patterns related to heart rhythm disorders. Fully connected networks or Multi-Layer Perceptron for the more structured clinical data: multiple risk factors and patient demographics to predict the outcome disease. By such different architectures, scientists can build robust models to enhance diagnostics' accuracy and patient management in cardiovascular healthcare.
**Convolutional Neural Networks (CNNs):** CNNs are particularly effective for analyzing medical images due to their ability to automatically learn relevant features from the data. They have been successfully applied for tasks like Detecting coronary artery stenosis in angiograms, Identifying abnormalities in echocardiograms.

**Recurrent Neural Networks (RNNs):** RNNs are well-suited for analyzing sequential data like ECG recordings or blood pressure measurements over time. They can capture temporal relationships within the data to predict CVD events like arrhythmias or heart failure.

**Deep Autoencoders:** These models can learn latent representations of complex data, allowing for dimensionality reduction and anomaly detection. They can be used to identify subtle patterns in EHR data that might be indicative of CVD risk. Through this survey, we hope to contribute to the growing body of knowledge on the integration of artificial intelligence techniques in cardiology, ultimately striving towards more precise, timely and personalized approaches to combat cardiovascular diseases.




**Related Works**

Rao, Gorapalli Srinivasa, and G. Muneeswari say the world's greatest cause of death is cardiovascular disease (CVD), especially for those with lower incomes and in environments with limited resources. In order to identify and treat CVD, medical professionals are using machine learning (ML) algorithms. The healthcare sector produces enormous volumes of data, yet effective methods for information extraction are few. ML methods are helpful in forecasting cardiac conditions, evaluating intricate medical data, and pinpointing practical and effective methods. The world's greatest cause of death is cardiovascular disease (CVD), especially for those with lower incomes and in environments with limited resources. In order to identify and treat CVD, medical professionals are using machine learning (ML) algorithms. The healthcare sector produces enormous volumes of data, yet effective methods for information extraction are few. ML techniques are helpful in assessing complicated healthcare data and forecasting cardiac problems. Ullah, Tahseen, *et al*. study offers a novel framework that makes use of optimum feature selection strategies and machine learning algorithms to identify and categorize cardiovascular illness. The framework assesses five distinct feature selection methods; FCBF performs best, combining with Extra Tree and Random Forest models to achieve an accuracy of 78%. This method works especially well with big datasets that mostly consist of categorical variables. With the help of prompt diagnosis and treatment, the proposed scalable machine learning-based architecture hopes to transform healthcare and lower the death toll from CVD. The Extra Tree and Random Forest classifiers in the machine learning framework achieve 100% accuracy in predicting patients' cardiac problems. This design may help patients receive better care and reduce mortality from CVD.

Cardiovascular issues are a major concern, and machine learning approaches have been used to decision-making systems in recent studies. To enhance algorithm performance, this work makes advantage of the heart disease datasets from UCI and Kaggle. It was suggested to use the TLV (Two-Layer Voting) model, an ensemble technique for both soft and hard voting. With an accuracy of 99.03% and 88.09%, the model surpassed previous research on the prediction of CAD illness by utilizing a combination of several algorithms and fine-tuning the classification methods. Cardiovascular issues are a major concern, and machine learning approaches have been used to decision-making systems in recent studies. To enhance algorithm performance, this work makes advantage of the heart disease datasets from UCI and Kaggle. It was suggested to use the TLV (Two-Layer Voting) model, an ensemble technique for both soft and hard voting. The model performed better than current.[3]

This work focuses on using machine learning approaches to the early diagnosis of cardiac disorders, including myocardial infarction. To enhance heart disease predictions, it makes use of seven classifiers: Random Forest, Gradient Boost, XGBoost, CNN, SVM, KNN, and LR. The model's diagnostic accuracy is improved when an XGBoost model is optimized, producing impressive outcomes like as 98.50% accuracy, 99.14% precision, 98.29% recall, and a 98.71% F1 score.[4] Pachiyannan, Prabu, *et al*. (ML-CHDPM) helps diagnose and categorize congenital heart disease (CHD) in expectant mothers with speed. The algorithm classifies instances according to clinical and demographic

parameters using cutting-edge machine learning techniques. An average of 94.28% accuracy, 87.54% precision, 96.25% recall, 91.74% specificity, 8.26% false positive, and 3.75% false negative are achieved by the ML-CHDPM.

**Problem Identification**

Some of the significant challenges for the usage of ML and DL models in CVD detection are their applicability in real-world settings. It is also concerned about the availability and the quality of the dataset. Most studies recognizethat obtaining large high-quality datasets is challenging; actually, this limitation becomes a challenge in training ML/DL models, and it has been found to be somewhat of an issue for the generalizability of their findings to a broader population. Another important issue is interpretability. Most of the ML and DL algorithms, particularly the deep learning models, seem to work like "black boxes"; thereby, it is difficult for clinicians to understand what is actually happening inside the models. Hence, it will be tough for clinicians to rely on such models and to assimilate their predictions into the clinical workflow decision-making process, as nobody could explain why the model makes those predictions.Integration of the new advanced models also raises a lot of difficulties from the aspect of integration of these new advanced models into the current clinical workflows. For these models to be appropriately adopted in healthcare, proper attention must be given to how they would fit into the present health provider practices. Training and possibly workflow adjustments are necessary to fit in these technologies in their use by clinicians. Again, some studies conclude for high accuracy, but generalizability of such models poses great concern. Accuracy can be very high on some specific datasets, but between different populations, the models don't work well, and that's particularly true if you make use of data that is typically nondiverse for the patient groups.

The problem in focusing strictly on some such specific datasets is that it puts severe restriction on the models applied to real-world patient populations, which are always more diverse. Ensemble methods, which combine multiple algorithms in a single method to achieve higher accuracy, have also not been explored much. These can be explored in a systematic way for enhancing predictive performance. Traditional ML approaches being applied more frequently in such studies, strengths of DL techniques on automatic image analysis have still remained underplayed in most research works related to CVD detection.Moreover, limited research has been carried out on the real-time integration of data, despite which some studies mentioned the use of real-time data streams from IoMT devices, but continuous development of such streams into CVD detection models is still a subject for further research. These types of issues therefore require continued research in improving the quality of the data gathered, ensuring model transparency, generalizability, and its integration into clinical settings so that ML and DL models related to the detection of CVD can reach their full potential.

## CONCLUSION

Machine learning (ML) and deep learning (DL) algorithms have emerged as very potent tools for the detection of cardiovascular disease (CVD). Recent studies demonstrated much promise within these techniques, especially regarding improvements in diagnostic accuracy and early prediction. This domain has spanned explorations in many algorithms including the traditional machine learning models of RF, XGBoost, SVM, and the advanced deep learning architectures include CNNs. The methods employed have proven to be especially effective for pattern discovery in medical data. Predictions achieved often surpass 98% accuracy in some studies.However, the success and generalization of these models depend a lot on the quality and representativeness of the datasets they are trained from. Most studies directly use datasets from sources such as UCI or Kaggle, which may not be representative of real-world populations. This makes the models highly data-dependent and limits generalization across demographics, making them less effective for a wider application in clinical settings. This results in many models being developed with general applications, such as predictions of cardiovascular diseases. Other models are specific in terms of their detection, as with congenital heart disease. These target applications serve to further emphasize the flexibility observed with ML and DL models applied in healthcare but reveal a need for the precise datasets to be utilized in addressing various clinical needs.This lack of interpretability forms one of the significant challenges in the application of sophisticated algorithms, such as deep learning. Many complex models can be said to be "black boxes,

"in that it is impossible to understand how they arrive at their respective predictions. That becomes a challenge because opacity, if not explained, could very easily lead to trust issues when such models begin to find applications in clinical settings where lack of transparency and explainability might raise questions regarding the resultant decision made by the professional practitioners themselves. Where the ML and DL models excel in terms of accuracy, interpretability is an issue that faces these models, which, if not overcome, should not be merged into healthcare applications.

**Future Research Should Focus On**

Improving data quality and access for training and validating models.Developing interpretable models to enhance trust and transparency in clinical settings.Investigating the integration of these models into existing clinical workflows.Exploring applications beyond traditional risk prediction, such as personalized medicine and real-time patient monitoring.Overall, ML and DL offer powerful tools for CVD detection. By addressing the challenges and continuing research, these techniques have the potential to significantly improve cardiovascular health outcomes.

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**Table 1: Comparison of different algorithm used to predict cardiovascular disease**

| S.no | Author Name & Year of publication | Algorithm Used | Advantages | Disadvantages |
|---|---|---|---|---|
| 1 | Rao, Gorapalli Srinivasa *et al* (2024) | Machine Learning and Data Mining | - Large amounts of data available | - Data quality and privacy concerns |
| 2 | Ullah, Tahseen, *et al*. (2024) | Feature Selection + Extra Trees / Random Forest | - Achieves 78% accuracy | -Limited generalizability if data not representative |
| 3 | Omkari, D. Yaso, *et* | Two-Layer Voting (ensemble) | - Achieves 99.03% accuracy | - Relies on pre-existing datasets, may not |

| | | | | |
|---|---|---|---|---|
| | *al* (2024) | | | reflect real-world variations |
| 4 | Ogunpola, Adedayo, *et al*. (2024) | XGBoost, Random Forest, Gradient Boost, CNN, SVM, KNN, Logistic Regression | - Improved diagnostic accuracy (XGBoost: 98.50% accuracy) | - Difficulty interpreting complex models (XGBoost, CNN) |
| 5 | Pachiyannan, Prabu, *et al* (2024) | ML-CHDPM | - Fast and accurate for congenital heart disease (CHD) prediction | - Limited to specific application (CHD) |
| 6 | Singh, Sanjeev, *et al* (2024) | Deep Convolutional Neural Network (DCNN), Recurrent Neural Networks (RNNs) | - High accuracy (DCNN: 99.42%) | - Requires large datasets for training |
| 7 | Fathima, A. Jainul *et al* (2024) | Not specified (mix of ML and DL algorithms) | - High accuracy reported with ensemble methods (99%) | - Limited information on specific algorithms and datasets used |
| 8 | Ainiwaer, Aikeliyaer, et al. (2024) | VGG-16, 1D CNN, ResNet18 | - Achieves good sensitivity (80.4%) and specificity (86.2%) for CAD detection | - Limited data source (heart sound recordings only) |
| 9 | Hammoud, Ahmad, *et al* (2024) | Random Forest, Gradient Boosting Classifier, KNN, SVM, Decision Tree, Logistic Regression | - Achieves high accuracy with Random Forest (92.85%) | - Difficulty interpreting complex models (e.g., Gradient Boosting) |
| 10 | Mohsen, AyedhAbdulaziz, *et al.* (2024) | k-Nearest Neighbors (k-NN), Support Vector Machine (SVM), Naive Bayes | - High diagnostic and classification accuracy reported | - Limited information on specific algorithms and datasets used |

**Table 2, fig 1: Accuracy details of various algorithms used to predict cardiovascular disease**

| Reference | Algorithm | Accuracy |
|---|---|---|
| Ullah *et al*. (2024) | Feature selection+ Machine Learning | 78% |
| Ogunpola *et al.* (2024) | XGBoost (highest) | 98.50% |
| Omkari *et al.* (2024) | Two-Layer Voting | 99.03% |
| Singh *et.al.* (2024) | Deep Convolutional Neural Network | 99.42% |

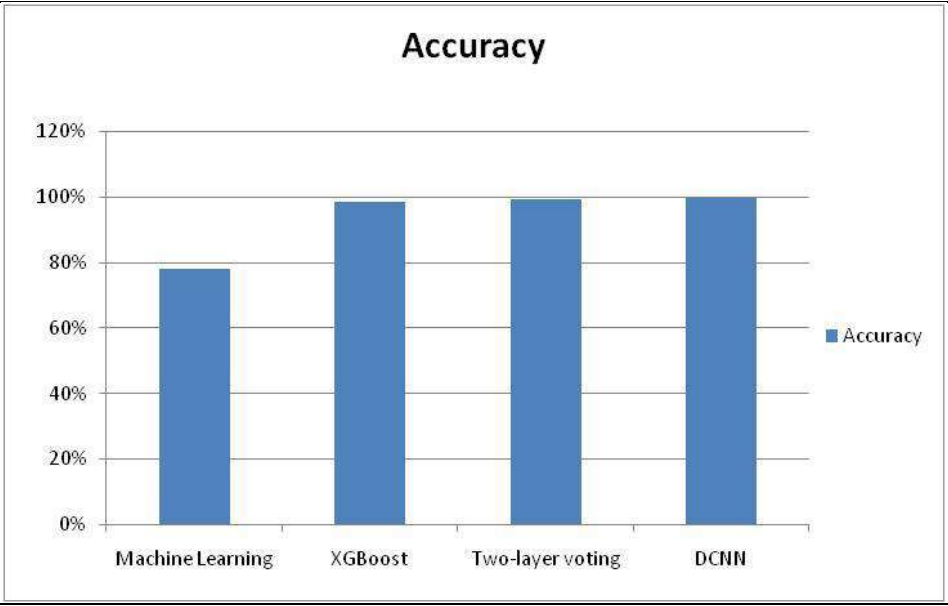


**Figure 2: Bar Chart on Comparison of Algorithm Accuracy in Cardiovascular Disease Prediction**

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**REVIEW ARTICLE**

# Recent Advances in Cloud Computing Security Protocols: A Systematic Review and Analysis

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## ABSTRACT

This review discusses the latest developments in methods to ensure security for cloud computing platforms, particularly in the areas of authentication, data authenticity, and preserving the privacy of cloudstored data. A study of ten disparate protocols draws out different ways of improving cloud security such as blockchain, lightweight authentication, contract-based realization, dynamic audit paradigm, and architecture integration. The main trends are outlined as distributions in favor of blockchain technology for more reliable and maximally decentralized applications, lightweight cryptography for utilization in constrained environments, and multi-cloud architectures for maximally comfortable and highly effective applications and services. Analyses conducted in the preceding sections compare a current strategy with representative previous techniques commonly used to implement a detailed security protocol and indicate that even when compared to development made just two years earlier most of the protocols provide better security features and more resistance to typical attacks, But, threats on the model implementation, modeling the massive scale, and examining the actual functionality of the model is also identified. While some protocols provide high theory-level results, many protocols have not received full-scale practical testing, which brings the issue of their functionality in largescale cloud environments under question. The conclusion can be derived from the fact that despite coming up with new protocols with distinct concepts of solving key issues of cloud security, widespread implementation in real environments and standardized evaluation are pending.

**Keywords:** Cloud Computing Security, Authentication Protocols, Data Integrity, Privacy Preservation, Blockchain, Cryptography, Multi-Cloud Environments, Dynamic Auditing, Lightweight Authentication, Scalability

# INTRODUCTION

Cloud computing has brought a great change in organizations' storage and computing process of data, due to its flexibility, scalability, and reducing costs. However, migration to cloud services has also brought new security issues especially when it comes to the aspects of authentication, data consistency, and privacy. While the cloud is necessary for organizations to incorporate reliable measures to enhance the security of the occupied cloud and data This is a review paper that will analyze the existing literature on recent trends in cloud computing security protocols that were proposed between 2020 and 2023. We dissect ten different methods whose aim is to solve ten different concerns of cloud security such as authentication, data integrity, and privacy-preserving technologies, among others. These protocols are among the modern approaches for increasing cloud security based on methods like blockchain, lightweight cryptography, multi-cloud platforms, and dynamic auditing models. The goal of this review is to survey recently developed cloud security protocols and their technologies and to compare and contrast these methodologies' effectiveness in addressing certain security issues.The protocol discussed in the present work is as it covers almost all the aspects of security in cloud computing from identity and access control systems using blockchain to efficient authentication protocols for mobile computing cloud some target improvement of data integrity checks in the multi-cloud environment and others suggest the approaches to vulnerability detection or secure data transfer. Also, the first protocols needed for heterogeneous systems are to be reviewed, integrated solutions based on blockchain, as well as efficient mechanisms for public auditing.The presented approaches are diverse due to the complex nature of the problems cloud security embraces and the constant process of creating new methods that are more protected, efficient, and realistic. All of them have specific goals in mind, ranging from the reduction of some weakness or enhancement of a particular method, and most of the time, they engage advanced technologies or otherwise, unexplored approaches, based on the integration of previously applied methods. Thus, the analysis of these protocols will help to reveal the current trends in cloud security research and to determine the perspectives for further investigation. This review will also explain how to address this gap in knowledge by identifying new approaches that are being used to improve cloud security and the remaining issues that will challenge researchers and practitioners in this dynamic field.

So, we anticipate that the systematic review will advance the discussion on cloud security and turn out to be beneficial for scholars, programmers, and corporations who want to improve their cloud security. In this way, the review may guide the subsequent research agendas and contribute to establishing improved, effective, and easy-to-use security measures that may protect the data in cloud computing.

**Overview of the security protocols**

**What security protocols and their role in cloud computing**

Security measures in cloud computing as exemplified by the set collection of papers include one or more systems of rules and practices, procedures, and cryptographic tools aimed at safeguarding the data, communication, and resources in a multiple cloud environment. These protocols act as the basis of the protection of information and services available and accessed in cloud environments. Cloud security is vital and has various functions, which are best illustrated by the given protocols. These protect information that is to be stored in the cloud by preventing unauthorized access, modification, or leakage of that data at different stages of storage in the cloud environment. These protocols help to create trust between different participants in the cloud environment and allow a secure exchange of information in conditions when resources are common and accessed almost physically. From the papers presented it can be seen that today's cloud security formats can tackle numerous issues. Among them, some of the major problems solved by them include identity management, access control, verification of data integrity, secure authentication, vulnerability detection, and the compatibility of two or more cryptosystems. The protocols use diverse sophisticated technologies and strategies such as blockchain, machine learning, lightweight cryptography, and ones that integrate two or more security measures. One commonality that can be seen in all of these papers is that more security and better performance are not mutually exclusive goals. Some of the proposed protocols are described to improve security while at the same time, inflicting minimal performance penalty. This is especially so in

cloud computing scenarios where issues to do with flexibility and resource utilization are of paramount importance. Another factor that can be seen here is how well these protocols could be applied across different cloud environments. It extends to multi-cloud architectures, and mobile cloud computing and has incorporated different security measures tailored for each kind of cloud deployment mode and services. This flexibility is paramount within the existing cloud computing context which is heterogeneous by nature and constantly evolving. The papers also stress the increase of preventive security activities' significance. Such measures like the Vulcloud protocols are proactive as opposed to reactive in that they are designed to search for weaknesses before they are exploited as is the case with most cloud security postures. These protocols have to do with issues of clarity and traceability in the management of cloud security. Incidences like third-party auditing and other reliable methods that can be used to validate the information further enhance confidence and adherence to regulations. In conclusion, the approaches to security presented in these papers put together a strategic plan for cloud security. Several operations are included in them; for instance, encryption, authentication, authorization, data integrity, and intrusion detect.

**The criteria for evaluating these protocols**

According to the cloud computing security protocols, any evaluation is always marked by a set of criteria that defines the eligibility of the security protocols based on several factors that include the feasibility of the protocols and the appropriateness of the same in the different cloud environments. These criteria, as evident from the collective research presented in the papers, encompass several key areas. Security characteristics are at the core of the assessment; the emphasis is made on the resistance of the protocols against different kinds of attacks. This entails evaluating the level of protection that a particular B2B eCommerce platform provides against typical threats like MITM attacks, impersonation, and unauthorized modification of the data. In this case, factors such as the strength of the use of encryption algorithms in giving secure and authentic means of accessing data, and the capability to provide data integrity are important. For example, the features of employing blockchain as a part of BC-IAS protocol are examined to determine whether it offers provable safety against malicious attempts to alter the data; another example is the examination of the Novel Pairing-Free Lightweight Authentication Protocol for its effectiveness in withstanding specific forms of attack, such as the stolen smart card and the disclosure of session-specific temporary information leakage. Another important factor is efficiency because cloud environments operate with limited resources and highperformance requirements. This is in terms of the amount of time taken for computation, the time needed to transfer data from one device to the other, and power consumption. It can be observed that all the papers underscore lightweight operations and the low computational complexity of the algorithm. For instance, the Dynamic Level-Based Integrity Checking Protocol (DA-ICP) its effectiveness relies on probabilistic proof of possession; checking possession of complete datasets is much more time-consuming. This is because large numbers of users are expected to be supported starting from small numbers and similarly where large volumes of data and information are anticipated. Evaluation of protocols is based on the criteria related to the sustainability of the performance and security at higher levels of operational scale. For example, the identification protocol for the heterogeneous systems should be examined by its ability to operate in different cryptosystems to solve the scalability problem of various cloud systems. Another key criterion is called flexibility and adaption or, in other words, the ability to change behavioral patterns. They outline how the protocols perform in support of data dynamic operations as well as across multiple clouds and multiple use case scenarios. For instance, the Dynamic Reciprocal Authentication Protocol is examined based on how efficient it can be when applied to mobile cloud computing to show how adaptable it is when implemented in certain environments. These are features such as latency, throughput, and response time that are viable measures of evaluation of the system. These are beneficial in that they assist in determining the effectiveness of the protocols on overall cloud service provision. Some papers present increased data access rates and decreased end-to-end delays as such performance indicators. Usability and ease of implementation can also be counted as other important assessment criteria. Where a protocol is quite hard to implement or manage, the protocol may not work well when adopted in the real world. For instance, when analyzing the ECC-based Authentication Protocol, an important criterion used is how simple the implementation is and how it can work in real-life situations. The following are other evaluation criteria; compliance with the industry standard and legal requirements. Legal and regulatory conformance refers to the evaluation of protocols based on legal and regulatory requirements including data protection laws and other industry standards. Those aspects of the protocol

that can be audited and the level of transparency are assessed, especially where the protocol models the ability to be audited or contains provisions for external auditing and the checking of data integrity. All of these are instrumental in creating confidence in the use of cloud services and they help in establishing responsibility. Practicality is one of the measures, where the importance of a given security measure is balanced against the costs of deployment and management. The protocols are sometimes compared and assessed on their innovative features addressing future security issues of the cloud computing environment. Part of this is evaluating new ways such as the application of the blockchain system in BC-IAS or the integration of several analysis methods in the Vulcloud Protocol. These evaluation criteria present a coincidental framework for the evaluation of the suitability and efficiency of security protocols in the cloud environment. This is a clear indication of the current struggle to meet physical security and at the same time ensure service delivery and reasonable use of cloud security systems.

**Protocols Reviewed**

**Blockchain-based IAS protocol to enhance security and privacy in cloud computing**

The BC-IAS protocol that was created by S. Navin Prasad and C. Rekha in 2023 concerns novel ways of increasing security and privacy in cloud computing. In the context of cloud computing, this solution is related to the principal domains of security, namely identity and authentication, access control, and confidentiality. The idea on which the BC-IAS protocol relies is that blockchain has inherently good characteristics which makes it highly secure and decentralized. This approach greatly enhances the general security position of the cloud computing platforms against many of these cyber risks. The general idea of the protocol is to enhance several key performance factors, including rates of getting data plus ratios of message deliveries plus the time it takes for the whole journey from one node to the next node, which is of paramount importance in cloud computing.

Related to the first, one of the most valuable aspects of the BC-IAS is its capacity to sustain high performance despite growing values of system faults. That is why this feature is especially important in distributed cloud networks where nodes can fail, and networks can have problems at any moment. Further, the protocol retrieves fairly good energy performance, which is crucial given the modern topic of green IT. But as with many decentralized applications based on blockchain, BC-IAS also has its drawbacks. The integration of such a system adds another level of difficulty to the currently complex cloud structures, thus giving organizations that wish to employ this technology a hard time. It is also not out of the doubt to entertain issues of possible performance penalties of the type incurred by general blockchain motion, especially at large-scale, enterprisestandards.The example of BC-IAS shows another problem that is typical for the majority of blockchain technologies, along with the scalability one. In the case of large numbers of users or transactions, the management of the system and its ability to promptly meet customers' needs can be problematic. Furthermore, the use of consensus mechanisms may require resources that are more expensive as well as the need for the purchase of better hardware to support the chain's functioning, and this might cause high energy consumption in some cases.

However, these are the potent challenges that still cloud service has to face, yet the BC-IAS protocol has great prospects in the sphere of cloud protection. Blockchain, it's capable of bringing into view the likelihood of decentralized systems in encouraging data security and confidentiality in areas like cloud computing that are central to security vulnerability. As cloud security emerges, we may expect that the protocols such as BC- IAS, provide guidelines for the future development of cloud computing systems that are safe, effective, and dependable

**An efficient and secure protocol for checking remote data integrity in a multi-cloud environment**

The Dynamic Level Based Integrity Checking Protocol (DA-ICP) that was put forward by H. Anwarbasha and S. Sasi Kumar along with D. Dhanasekaran in 2021 has also brought a major enhancement in the field of data security in multi-cloud systems. This protocol aims at filling the existing gap where organizations are struggling to determine the authenticity of data stored in different cloud storage systems that exist in the organization's cloud environment due to the adoption of the multi-cloud model. Another factor that is critical to understanding the application of DA-ICP is that security is one of the most important issues determining cloud success and DA-ICP incorporates it

through the use of public key cryptography and EPDP encryption. They also apply the principles of confidentiality and integrity of outsourced data and present a good firewall against threats. Linear congruential generators used by the protocol for producing random sequences also add a level of efficiency to the general performance, which ultimately places this protocol among efficient ones.Another feature I like in DA-ICP is that the organization achieved high accuracy in the verification of data integrity with an accuracy of as high as 96%. 78%. The high degree of accuracy together with a shorter time to execute than currently existing methods makes DA-ICP a strong proposition that can help organizations that are trying to ensure data accuracy in multi-cloud systems. The protocol also provides a reasonable level of stringency of the verification rules simultaneously providing reasonable update times.

Although DA-ICP has several benefits, there are some disadvantages as well. Some potential issues of the protocol include: In some circumstances, the complexity of the protocol due to the scheme's multiple cryptographic operations and significantly detailed steps might present difficulties in terms of actual implementation as well as add to computational costs. Others argue that the protocol is best suited for a small number of data and is not ideal for huge data sets or when performing several analyses of data within a short time. The use of third parties for auditing while helpful for security opens an avenue of attack in case the auditors are hostile or have been compromised.
Moreover, the demonstrative results of the protocol in actual multi-cloud environments have not been verified yet, although the actual proof-of-concept picture in the paper is a mixture of theoretical and simulation outcomes. As acknowledged, the reality of the experimental results is that they were obtained from simulated and proof of concept environments only; one would naturally ask how DA-ICP may fare under different network conditions or potential issues arising in the real multi-cloud environment. However, all these notwithstanding, the proposed DA-ICP protocol is a big stride towards meeting the inherent practical imperative of data integrity check-ups in a stylized, complex cloud storage environment.

**A Novel Pairing-Free Lightweight Authentication Protocol for Mobile Cloud Computing Framework**
The paper titled 'A Novel Pairing-Free Lightweight Authentication Protocol for Mobile Cloud Computing Framework' developed by Azeem Irshad, Shehzad Ashraf Chaudhry, Osama Ahmad Alomari, Khalid Yahya, and Neeraj Kumar published in 2021 proposes a new direction for the issue of authentication in the concept of mobile cloud computing. The authors have set a protocol that has efficiency and security aspects and is specially designed for mobile devices.One of the key features of this protocol and, at the same time, its defining characteristic is the fact that it is essentially free of pairing. Due to the elimination of computationally costly bilinear pairing operations, the authors have come up with this much more efficient solution, which however incorporates all relevant levels of security. The uses of the protocol include user anonymity, where nobody can trace the identity of the other party, mutual authentication, and the ability to counter different forms of attacks such as impersonation attacks and stolen smart cards. It also provides perfect forward secrecy, in which even if the private key is compromised, the session keys will not be discoverable.Unlike other similar protocols, this one has the advantage of scalability, which is implemented so that it is easy to add a new user or a new server into the system without any changes to the smart cards or the repositories in the servers. This together with the elimination of the verifier table at the server end makes this feature very effective enhancing the practicality of the protocol. The authors have also done a formal security analysis on the protocol in question to prove the kind of security the protocol provides in theoretical formalism.

But there is always a but: the suggested protocol is not without some flaws. It works with lower computational cost than pairing-based schemes though it has a higher computational cost than some of the most lightweight security schemes. On this account, the authors justify this trade-off as making room for enhanced security features. The protocol also includes slightly elevated communication overhead as compared to some other schemes could be a problem in some Mobile environments having limited bandwidth.It is not clear though how easy or difficult it will be to implement the protocol given the number of steps and calculations involved compared to the simpler but less secure schemes. This could also add to the risk of implementation error was perhaps not well coded and tested as was the case with its predecessors. Also, the paper is about the protocol that is proposed for mobile cloud computing,

but the authors do not present any experimental results based on real mobile devices, and that might show new problems in the real-world usage of the protocol.

Therefore, this novel authentication protocol appears to be a viable way of having better security across mobile cloud computing while at the same time having superior outcomes in terms of efficiency. Although there are some shortcomings in this approach, especially in the aspect of implementation and performance it covers some basic security issues on MCC and can be implemented on low-end devices.

**Design of Hybrid Authentication Protocol for High-Secure Applications in Cloud Environment**

The paper 'Design of Hybrid Authentication Protocol for High-Secure Applications in Cloud Environment' written by Sai Srinivas Vellala and Dr. R. Balamanigandan was published in 2022. The need for this protocol stems from the increasing security risks associated with cloud computing, particularly for applications with high-security interests. In their papers, the authors suggest that a blend of different types of authentications can be even more effective, and prevent vulnerabilities in an overall system. The paper thus shows that the proposed hybrid protocol outperforms both the elemental authentication and the MFA protocol in most of the performance parameters. That is why the authors say that the protocol guarantees 95% security levels which can objectively be considered as a significant upturn in contrast with 64% in the case of basic authentication and 86% in the case of multifactor one. One of the significant issues with applications working in the cloud is data security and this aspect has been boosted by this innovation.

Another feature of the paper is the recount of the increase in overall system performance at various levels of the business. The hybrid protocol has been reported to offer 98% performance, thus presenting a slight improvement over the 86% for basic authentication and 91% for the multifactor one. This implies that it is possible to enhance the security of the cloud by agreeing on a protocol that still does not affect the computation speed which is a critical factor in the cloud in that many users and applications share the resources.

Another area in which the hybrid protocol appears to bring a major enhancement is in the concept of error reduction. The authors argue that their approach has the least errors at 8% while basic authentication has 73% and multifactor at 54%. Such a reduction could make cloud services more accurate and reliable, and thus potentially decrease incidences of maintenance downtimes, therefore increasing the satisfaction of users. Issues such as scalability, which is important in cloud computing since resources have to change in relationship to demand, are also solved by the protocol. On the subject of scalability, the authors note a corresponding figure of 87% as against 43% of basic authentication and 68% of multifactor authentication. This could help the protocol to scale up better in new and more active cloud environments with an increasing number of users and volume of data.

However, despite these impressive claims, the paper appears to have a significant limitation: the absence of some practical experiences obtained from the testing of the suggested proposals in a realistic cloud setting. Due to the scarcity of practical implementation data, the real efficacy and feasibility of the hybrid authentication protocol will remain dubious. Failure to incorporate actual implementation methods or someday research tests may hamper other researchers or practitioners from being able to authenticate the results or follow the outlined process on their own. The absence of practical testing also leaves several questions unanswered, for example, what major or minor scalability problems are to be expected in large-scale implementations of the protocol? Are there some previously unknown vulnerabilities that may become apparent only when the protocol is being used? What difficulties are to be expected when integrating the described protocol with existing cloud systems? These are key factors that should be taken into account in any new security protocol, more so one that is being designed to meet high-security usage.

Therefore, even though the theoretical enhancements discussed in this paper are quite substantial and might denote a breakthrough in cloud security, one can single out the lack of empirical correspondence as the major drawback. Further development on this proposed protocol would be even advantageous if empirical tests of the announced

enhancements are conducted in real cloud infrastructures rather than simulations; and if the possible issues that may appear in practice are tackled. Rather, this would provide a fuller picture of the protocol and its ability to solve the security issues present in the cloud and be ready for their high-security applications.

**Vulcloud Protocol**

Design a New Protocol for Vulnerability Detection in Cloud Computing Security Improvement

The paper "Design a New Protocol for Vulnerability Detection in Cloud Computing Security Improvement" was prepared by Narendra Mishra, R K Singh, and S K Yadav in 2021. They presented this protocol with the view of developing a new approach to the detection of a vulnerability in the existing security of cloud computing. The authors put forward a set of features and a set of recommendations for employing a static/dynamic Hybrid Analysis enhanced with Machine Learning Algorithm as a systematic and effective approach to discovering the vulnerabilities typical of the utilization of Cloud Computing.

Vulcloud aims to work effectively and combat the problems associated with virtualization and issues residing within cloud solutions. The current laboratory protocol uses multiple techniques of analysis in the process to achieve better vulnerability detection to improve cloud security. According to the authors, this type of approach to the problem yields a higher performance compared with conventional approaches, although no particular evaluation criteria are specified in the abstract. The flow that has been embodied in the Vulcloud Protocol has been partially automated. It contains some machine learning components that are derived and tested in a way that requires minimum or no manual input in the vulnerability detection process. This protocol will also attempt to further apply more symbolic execution to improve the detection of bugs in virtualization applications and demonstrate an attempt to tweak existing security paradigms toward the cloud. That said, this paper seems to have some of the following drawbacks. This raises some questions as there is very little implementation information there being only a general outline on the parameters of the protocol. It is not easy to prove much of the averred improvements over existing methods because the work offered lacks quantitative data or comparison figures. In addition, it is difficult to verify the implementation of the ideas behind the Vulcloud protocol in the real clouds or provide references to the cases of its effectiveness. Some of the overlying that the hybrid approach involves several techniques might make the implementation and the subsequent maintenance of the approach slightly complicated in some way. One more thing that the paper fails to do is to consider the possibility that this more complex protocol will take more time, or computing power, to implement, which is a concern when it comes to cloudy environments. There is also no information on specific practical concerns such as the approach taken by the protocol in differentiation between false positives and false negatives in the identification of weakness. The rendering of the protocol appears to be especially concentrated on virtualization risks, which could mean that lesser significant sorts of cloud-based threats may be disregarded. The paper does not explain to what extent the protocol is scalable for large clouds, nor does it include a direct comparison of the protocol to vulnerability detection tools prevalently used in cloud environments. The lack of a clear threat model and very little coverage of vulnerability mitigation is also missing here.

In conclusion, having analyzed the Vulcloud Protocol as a model for vulnerability detection in cloud computing, one has to admit that, although the idea is promising, the absence of detailed descriptions of the implementation of the actual detection model, of comparative numerical results demonstrating the efficiency of the approach, and information on real experiments with the Protocol make it very hard to judge the practical usefulness and effectiveness of the protocol. Future work should include more extensive tests of this protocol, the ways of its performance measurement, and a broader view of the range of cloud security threats rather than virtualization.

**Dynamic Reciprocal Authentication Protocol for Mobile Cloud Computing**

The paper entitled, ''Dynamic Reciprocal Authentication Protocol for Mobile Cloud Computing '' was authored by Abdulghani Ali Ahmed, Kwan Wendy, and Muhammad Nomani Kabir in 2020. The purpose of this protocol is to improve the security of communication in the context of MCC. It is for this idea that the proposed protocol seeks to counter the existing authentication challenges for a safe mutual authentication technique immune to known forms of

attack. The strengths of the protocol are in the security aspects and performance since they are improved. It uses identification with several factors, the method of Diffie-Hellman, and passwords for one-time conversations between mobile clients and cloud servers. Due to this aspect, the mentioned protocol is immune to several attacks including the man-in-the-middle, playback, impersonation, and synchronization attacks. Thus, the author concludes that the proposed protocol is computationally less demanding compared to other methods that can make it useful for data authentication and security of data communication in the context of mobile cloud computing. Another interesting aspect is related to the dynamics of the given protocol and relative improvements in usage. It uses dynamic passwording and is not susceptible to social engineering and is, therefore, a very reliable and easy-to-use authentication. It also guarantees the creation of correct session keys at both communicating parties for proper session management, and to minimize on leakage of session keys or insecure sessions.

However, the paper has the following limitations: Its strength lies in the fact that it aims and works solely on presenting and assessing an authentication protocol for mobile cloud computing and may not be adequate in addressing all the security aspects or all the security issues in the broader field of cyber security. Even though the paper offers metrics for the comparison of the proposed protocol with the previous works, there might be more metrics or points of view that could contribute to the evaluation. Another limitation of the paper is that there seems to be inadequate discussion on the deployment of the proposed protocol in real-life mobile cloud computing scenarios. However, the integration of such protocol as the Dynamic Reciprocal Authentication Protocol demonstrates some of the promising features that would ensure better security in the context of MCC hence more studies in the applicability of the actual implementation of the protocol, scalability, and user interaction in reality for better real-life understanding of the performance.

**Identification Protocol Heterogeneous Systems in Cloud Computing**

The paper "Identification Protocol for Heterogeneous Systems in Cloud Computing" by Srinath Venkatesan in 2022 offers a novel solution to handle with complexities of identification for heterogenic cloud networks securely. Of all these protocols, this protocol was unique in its exactness and versatility in the search between cipher texts that encrypted under different cryptosystems, namely CLC and IBC. This feature makes the protocol very practical for real-world cloud settings where at times one or more cryptographic systems are used. The protocol utilizes the Twisted Near-ring Root Extraction Problem to provide its security base, which is a great mathematical problem. One is that it provides better efficiency in preserving privacy by permitting the search of encrypted data without the leakage of information about the plaintexts is very important in the cloud. The authors say that the protocol is immune to concurrent active attack and impersonation attacks adding more credence to prevention.

The current protocol's most important advantage is best captured by the title of the work in question – an efficient and secured Identification Scheme. What is left theoretically as protection for the scheme is the Bilinear Diffie-Hellman assumption, which gives a sound theoretical ground for each of the security claims. But the protocol also has certain advantages that have been criticized as follows: It's deep-rooted in concepts such as near-rings and endomorphisms As such, it may be hard to apply and grasp by most people. Surprisingly, one gets a feeling that most of the paper is conceptual since the authors do not provide any account of how the purported techniques work in real-world cloud environments. The security proof of such schemes, namely the use of the random oracle model which is well understood to have its limitations in real-world usage, may not be a perfect match in terms of security.
Although it was mentioned that the approach is efficient, it can be seen that due to the numerous mathematical computations involved the strategy could possess a high level of computational complexity while being implemented in large-scale cloud environments. Nevertheless, the paper fails to provide a relative analysis of other protocols to fairly measure their performance and efficiency. There are also very few discussions pointed to key management and its integration into the existing cloud infrastructures which are crucial for the real-life implementation of the system.

Thus, the identification protocol proposed in this paper is an insolent and possibly effective solution in the context of heterogeneous clouds. The following are its advantages: security, privacy preservation, and theoretical efficiency. But knowing its flaws, it is still not without issues; it has not been used in practical implementations and the underlying maths is complex. Future work is recommended to be based on practical regard, efficacy comparisons with current solutions, scalability challenges, and effective use of the protocol in cloud computing.

**A Secure User Authentication Protocol Based On ECC for Cloud Computing Environment**

The paper 'A Secure User Authentication Protocol Based on ECC for Cloud Computing Environment' by Diksha Rangwani and Hari Om published in 2020 provides details of a cloud computing user Authentication using Elliptic Curve Cryptography. It meets several critical security concerns in cloud computing but also allows for high performance. It improves security because it addresses exposures that were observed in prior protocols, including DoS attacks, privileged insider attacks, stolen smart cards, and attacks. Its effect is to reduce the computational and communication costs which are important in efficient cloud computing with the use of dynamic and lightweight cryptographic hash functions. The security of the given protocol is shown by a formal security analysis of the Burrows–Abadi– Needham (BAN) logic and an informal analysis of attacks on the protocol, however, the strong point of this protocol is that it gives much consideration to users' privacy. It conceals the user's identity whilst authenticating and this is important in avoiding user tracking by intelligent adversaries. The analysis of comparison presented in the context of this paper reveals that it is more efficient than the current trends regarding the communication complexity, computational load, and storage required for the implementation of this protocol in real-world environments. Also significantly, the simpler means the easier it is to implement a protocol, and this enhances its applicability to cloud settings.

However, the protocol also has some limitations. The use of the ECC system brings improvements in productivity and security but may present problems in those places where this cryptographic method is not used or is not well known. ECC's enhanced basis of mathematical operations might entail professional competence for its implementation and management. They may also be associated with first-time costs for setup and overhead in large-scale clients. As with any new protocol, one always runs the risk of new weaknesses appearing as the members adapt to it over time, thus making constant review of the protocol essential. Therefore, this ECC-based authentication protocol is a promising solution for providing secure user authentication in a cloud computing environment. Security-wise, it outperforms other methods; in terms of efficiency and user privacy, it will be a good addition to the existing approaches. The potential implementers should be aware of the problems that arise concerning the adoption of ECC, and the necessity to engage professionals if the equipment and the software are to be properly operated. The study's limitations make suggestions for future work including addressing the limitations of this protocol about durability and examining the capabilities of this protocol on multiple real cloud environments.

**An Integrated Architecture for Maintaining Security in Cloud Computing Based on Blockchain**

The article "An Integrated Architecture for Maintaining Security in Cloud Computing Based on Blockchain" by Ruba Awadallah, Azman Samsudin, Je Sen The, and Mishal Almazrooie provides a clear solution for how security should be implemented in cloud computing. This research work was published in 2021 where the author presented a scheme using multiple CSPs and a blockchain to solve significant security issues related to cloud computing, namely data integrity and confidentiality.

As has been mentioned, the proposed architecture has several advantages. It improves data purity since blockchain can detect the tampering made, even by the CSPs themselves. Homomorphic encryption is used to maintain data security, while data operations can be performed on encrypted data. Distributing trust among several CSPs makes it difficult to have a single point of failure, thus the need to implement the scheme. They make use of public ledgers such as the Bitcoin or Ethereum blockchain to store master hash values, to make the verification data tamper-proof. The architecture is friendly to this level of flexibility in that it is scalable and flexible enough to integrate with different numbers of CSPs and blockchain platforms.

However, the working of the scheme is not without its flaws. It can only alert of data tampering but cannot identify the actual records that have been tampered with as acknowledged by the authors. Of course, there are possible problems relating to synchronization and additional issues for more clients who must maintain cryptocurrency purses. The use of public blockchain brings in dependencies on another external network. Further, the study is mostly of the conceptual kind and thus does not propose actual applications of the tool under analysis or test it on real-life conditions, which creates some uncertainty about the tool's efficiency when applied in real-life cases.

However, the proposed architecture goes a long way in addressing the security issues currently in the use of cloud computing. Based on the concept of distributed and confidential multi-cloud, it can be considered that the multi-CSP approach based on blockchain technology can become a new direction for further research and development of cloud security. The scheme has great advantages for the increase of data integrity and its confidentiality without appreciable loss of scale or simplicity of implementation, which strengthens this research's contribution to the subject.

**An efficient data integrity auditing protocol for cloud computing**

The paper titled "An efficient data integrity auditing protocol for cloud computing" written by Dr. Neenu Garg from Chandigarh University, India, and collaborated with Prof. Seema Bawa of Chandigarh University and Dr. Neeraj Kumar of Thapar University, Patiala, India, in the year 2020, has proposed an Identity-based Public Auditing Protocol for the cloud environments. The described protocol also has several important benefits when it comes to cloud data integrity verification. The primary strength is founded on the provision of third-party auditors to offer the public an independent confirmation of the security of the cloud services. One of the advantages is the effectiveness of the protocol in the context of storage overhead; to provide services for a file block, a single authenticator is used. Besides, it supports dynamic data operations which include update, insert, and delete operations and data integrity which is very important in actual cloud applications.

From the security aspect, it is fairly sound: the protocol is shown to be secure under the Random Oracle Model and uses the Computational Diffie-Hellman Problem as a basis. It also meddles with the computational load that clients have to endure during the setup phase, thus coming off as less complex. Working on the prototype, the authors can testify to the practical effectiveness of the proposed protocol due to the comparison of the results obtained with the results of traditional protocols. Also, the protocol considers portable as well as mobile devices making it very flexible in its approach. However such a protocol has its shortcomings. Despite the positive impact of having Third-Party Auditors (TPAs) to check the veracity of claims made by its partners, it has drawbacks to data privacy and security. The auditing process involves the exposure of information and the protocol is only as secure as the TPA implementing it claims to be. These causes make up a potential single point of failure and can be a source of concern to clients of the TPA in terms of its impartiality. Some issues may potentially appear when dealing with increasingly large amounts of data and numbers of clients – scalability of the auditing protocol. In addition, the involvement of TPA enhances the increased process of managing the auditing process.

Finally, it has been demonstrated that this paper provides a new pathway to data integrity auditing in cloud computing scenarios which outperforms other approaches in terms of efficiency, security, reliability, and flexibility. However, there are potential disadvantages linked to privacy, trust to the auditors and third parties, and scalability of the solution.

## CONCLUSION

The following review of ten protocols shows that each of those areas has made important progress in security, including authentication, data integrity, privacy preservation, and vulnerability detection. Technologically, security is being boosted by such innovations as blockchain and machine learning, and in regard the industry trends, multiple

methods implemented on the cloud are being paid attention to. The concept of protocols is to act as a middle ground between security and performance, and as such the problem of scalability in practical settings is not fully solved. Most do not have thorough testing, which raises a question as to the applicability of the models that are studied. Integrated solutions and adaptive measures are possible as well as enhanced focus on user privacy of the systems. Implementation, standardization, and changes in threats again proved to be still a work in progress. More research and experiments are needed to work in this field and make stronger cloud security to achieve more people's confidence and adaptability of cloud computing in various industries.

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**REVIEW ARTICLE**

# Cyber Intrusion Detection: A Systematic Review of Data Mining and Machine Learning Methods for Improved Threat Identification and Response

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## ABSTRACT

Effective intrusion detection is essential for protecting digital assets as cyber-attacks grow more serious. The advantages and disadvantages of several strategies, such as supervised, unsupervised, and deep learning techniques, are highlighted in our review. Additionally, we go over how enhancing detection accuracy may be achieved by feature selection, hyperparameter optimisation, and data preprocessing. The review's conclusions offer guidance to researchers and cybersecurity professionals on how to create intrusion detection systems that are more resilient and adaptable, thereby fortifying cyber defences. The study offers a thorough analysis of the state of cyber intrusion detection at the moment, highlighting the difficulties and restrictions of present techniques. The paper emphasises the inadequacies and limitations of current methodologies and underlines the need for creative solutions through a rigorous evaluation of data mining and machine learning technologies. The research lays the groundwork for future developments and breakthroughs in cyber intrusion detection by identifying these difficulties, ultimately boosting the security and resilience of digital systems.

**Keywords:** Cyber-Attacks-Data Preprocessing-Digital System-Deep Learning-Supervised-Unsupervised.

## INTRODUCTION

The rapid growth of networked systems and the increasing sophistication of cyberattacks have made cyber intrusion detection a crucial component of contemporary cybersecurity. As cyberattacks grow more frequent and intense, organisations are finding it harder and harder to recognise them and take appropriate action. Modern technologies must be implemented in order to strengthen digital defences because the threat landscape of cyberattacks is becoming more and more dangerous, making traditional security measures insufficient. The necessity for creative solutions that can identify and adjust to changing threats has grown critical as the number and complexity of cyberattacks increase. Sophisticated threats are difficult to detect and counter using cyber intrusion detection, a crucial element of contemporary cybersecurity. Attackers now have an easier time exploiting new vulnerabilities that have been brought about by the increasing interconnection of devices and systems. To identify and counter these threats, conventional security techniques are no longer enough. More sophisticated technologies are required to improve threat detection and response, such as machine learning and data mining. Because cyberattacks are so complicated, creative solutions that can change with the threats are needed. Real-time threat identification and response is a critical component of cyber intrusion detection. There has never been a more pressing need for efficient cyber intrusion detection. For organisations to remain ahead of new risks, they must implement cutting-edge technologies.

More sophisticated and adaptable techniques are clearly needed as the conventional signature-based detection methods are no longer enough. Since cyberattacks are become more sophisticated, focused, and challenging to identify, it's critical to create cutting-edge defences against ever-changing threats. Data mining and machine learning techniques have emerged as promising solutions, offering the potential to enhance threat identification and response. These techniques can analyze vast amounts of data, identify patterns, and detect anomalies, making them well-suited for cyber intrusion detection. A thorough grasp of the present state of research is necessary because the discipline of applying data mining and machine learning to cyber intrusion detection is still in its infancy. With an emphasis on the benefits, drawbacks, and potential applications of data mining and machine learning techniques in cyber intrusion detection, this systematic review attempts to present a comprehensive summary of the body of research in the field.

By examining the existing literature, this review seeks to identify the most effective techniques, gaps in current research, and opportunities for innovation. This will ultimately inform the development of more resilient and adaptive cyber intrusion detection systems, capable of detecting and responding to emerging threats. In addition, this paper will examine the several approaches to data mining and machine learning—supervised, unsupervised, and deep learning—that are employed in cyber intrusion detection. Additionally, the significance of feature selection, hyperparameter adjustment, and data pretreatment for enhancing detection accuracy will be covered. This review will also look at the difficulties and constraints facing current research, such as problems with interpretability of models, class imbalance, and data quality. Researchers and practitioners can improve the security and resilience of digital systems by tackling these issues and creating cyber intrusion detection systems that are more effective.

## LITERATURE REVIEW

**1.**Farah Jemili, Rahma Meddeb, and OuajdiKorbaa, "Intrusion detection based on ensemble learning for big data classification," November 7, 2023. The assessment and comparison of machine learning algorithms, including Random Forest, XGBoost, and decision trees, is the main topic of this work. Interestingly, the result of our research is a hybrid intrusion detection model that combines the advantages of both techniques. Their investigations show that the hybrid model performs exceptionally well, especially when Random Forest and XGBoost are combined. In some situations, this method has an accuracy rate of 97%, outperforming individual methods. In conclusion, their research highlights the significance of ongoing adaptation in the face of threats and show how ensemble learning can improve

intrusion detection.[1]. 2.In 2024, Diptiben Ghelani published Enhancing Data Security: Machine Learning Approaches for Intrusion Detection in Computer Networks.The design ideas, essential elements, and assessment metrics of machine learning-based intrusion detection systems (IDSs) are covered in detail in this study. Additionally, it talks about the drawbacks and restrictions of ML-based intrusion detection systems, including the requirement for labelled training data, interpretability of the model, and flexibility in response to changing threats. The study also looks at new developments in machine learning, including deep learning and reinforcement learning, and how these might be used in intrusion detection. Through a critical analysis of existing research literature and case studies, the paper highlights the strengths and weaknesses of different ML approaches for intrusion detection, offering insights into their effectiveness, scalability, and practicality in real-world deployment.[2].

3.In 2024, Shereen Ismail, Diana W. Dawoud, and Hassan Reza conducted a comparative analysis of datasets with the purpose of detecting cyberattacks in wireless sensor networks.The two datasets are compared in this research based on their salient features, dataset quality, and machine learning classification performance. Recursive Feature Elimination (RFE) and Mutual Information (MI) are utilised in feature selection. The statistical information calculation is used to measure the quality of the dataset. Three supervised ensemble techniques—LightGBM, bagging, and stacking—have their ML classification performance examined. Evaluation criteria such as classification accuracy, model size, processing time, and probability of false alarm, misdetection, and detection probability are used.[3]. 4.In 2015, Anna L. Buczak and Erhan Guven published A Survey of Data Mining and Machine Learning Techniques for Cyber Security Intrusion Detection. A concentrated literature review of data mining (DM) and machine learning (ML) techniques for cyber analytics supporting intrusion detection is presented in this work. Each ML/DM approach has a brief lesson description supplied. Papers that represented each method were found, read, and summarised based on factors such as the quantity of citations or the significance of a developing technique. Some well-known cyber data sets used in ML/DM are given because data are crucial to ML/DM techniques. The article discusses the difficulties of utilising ML/DM for cyber security, the intricacy of ML/DM algorithms, and some guidelines on when to apply each method are provided.[4].

5.Rahul D. Shanbhogue and B. M. Beena's 2017 survey, "A Survey of Data Mining (DM) and Machine Learning (ML) Methods on Cyber Security," This paper covers the relevant basic information on cyber security and surveys the use of machine learning (ML) and data mining (DM) techniques in automating the cyber detection system. Results are Numerous Machine Language and Data Mining techniques have been used to address these problems after a variety of cyber intrusion detection and security vulnerabilities were identified. The analysis section of this study clarifies the promise, quirks, and complexity of using ML algorithms to cyber security. Utilisation: Real-world cyber security intrusion detection systems use the machine learning and data mining techniques and algorithms covered here.[5]
6.Priyesh Kulshrestha and T. V. Vijay Kumar's machine learning-based intrusion detection system for IoMT is scheduled for release in September 2023. In order to address this problem, this study presents machine learning-driven intrusion detection systems. The goal of this research is to develop an intrusion detection system (IDS) with an emphasis on machine learning (ML) that can detect cyberattacks against IoMT-based systems. Many machine learning (ML) techniques for classification were applied, including Multinomial Naive Bayes, Logistic Regression, Logistic Regression with Stochastic Gradient Descent, Decision Tree, Ensemble Voting Classifier, Bagging, Random Forest, Adaptive Boosting, Gradient Boosting, and Extreme Gradient Boosting. Based on performance metrics like accuracy, precision, recall, F1-score, False Detection Rate (FDR), and False Positive Rate (FPR), the best performing technique was found through experimentation.Furthermore, on performance criteria like accuracy, F1-score, FPR, and FDR, it was discovered that the adaptive boosting based IDS for IoMT outperformed the current ToN_IoT-based IDS models. [6].

**Challenges and trends**

Challenges in cyber intrusion detection using machine learning and data mining include the evolving threat landscape, which makes it difficult for models to keep up. Additionally, high-quality data is required to train models, but it can be difficult to obtain and label. False positives and negatives are also a concern, as models can generate false alarms or miss attacks, leading to wasted resources or undetected threats. Furthermore, the interpretability and

explainability of models can be a challenge, making it hard to understand why a particular decision was made. Finally, scalability and performance can be an issue, as models can be computationally intensive, requiring significant resources to run. Trends in cyber intrusion detection include the application of deep learning techniques, such as neural networks, to improve detection accuracy. Anomaly detection methods are also being used to identify unusual patterns in network traffic. Hybrid approaches, combining machine learning with other techniques, such as rule-based systems, are being explored to improve detection accuracy. Explainable AI is another trend, with researchers developing techniques to explain model decisions and improve interpretability. Transfer learning is also being used, where pre-trained models are fine-tuned for specific cyber intrusion detection tasks. Graph-based methods are being applied to analyse network traffic and detect intrusions, and real-time detection is becoming increasingly important. Finally, edge AI is emerging, where models are deployed on edge devices, such as routers and switches, for decentralized detection.[7].

**Limitations of cyber intrusion detection using ML and DM include**

**Data quality and availability issues**

Data quality and availability issues refer to the challenges of obtaining accurate, complete, and relevant data for training and testing machine learning models. Poor data quality can lead to biased or inaccurate models, while insufficient data can limit model effectiveness. Additionally, data may be difficult to obtain due to privacy concerns, proprietary restrictions, or lack of standardization. Noisy, inconsistent, or missing data can also impact model performance. High-quality data is essential for developing reliable cyber intrusion detection systems.

**Evolving threat landscape**

The threat landscape is constantly changing, with new attack vectors and techniques emerging daily. Attackers adapt and evolve their methods to evade detection, making it challenging for machine learning models to keep up. Zero-day exploits, polymorphic malware, and social engineering tactics are just a few examples of evolving threats. As new technologies and systems are developed, new vulnerabilities are introduced, providing opportunities for attackers to exploit. This dynamic environment requires continuous updating and refining of cyber intrusion detection systems to remain effective.

**False positives and negatives:**False positives occur when a system incorrectly identifies benign activity as malicious, leading to unnecessary alerts and wasted resources. False negatives happen when a system fails to detect actual malicious activity, allowing threats to go undetected. Both types of errors can have significant consequences, including system downtime, reputational damage, and compromised security. High false positive rates can lead to alert fatigue, causing security teams to ignore or miss critical alerts. False negatives can result in undetected breaches, data theft, or system compromise

**Lack of interpretability and explainability:**Machine learning models used in cyber intrusion detection can be difficult to interpret and understand, making it challenging to trust their decisions. The complexity of these models can lead to a lack of transparency, making it hard to identify why a particular alert was triggered. Without clear explanations, security teams may struggle to understand the context and severity of detected threats. This lack of interpretability can lead to delayed or incorrect responses to incidents. Furthermore, regulatory requirements and auditing needs may also be hindered by the inability to explain model decisions**.**

**Scalability and performance challenges:** Scalability and performance challenges are significant limitations in cyber intrusion detection. Machine learning models struggle to handle large volumes of network traffic and data, process high-speed data streams in real-time, and scale to accommodate growing network sizes and complexity. As a result, performance and accuracy decline, leading to reduced detection capabilities, increased false positives and false negatives, and delayed or missed detections. Furthermore, these challenges can lead to inadequate incident response and insufficient resource allocation, ultimately compromising the effectiveness of cyber intrusion detection systems. Addressing these scalability and performance challenges is crucial to ensure timely and accurate threat detection and response.

**Vulnerability to adversarial attacks and bias**

Machine learning models in cyber intrusion detection are vulnerable to adversarial attacks and bias, which can significantly compromise their effectiveness. Adversarial attacks, designed to evade detection or mislead the model, can exploit vulnerabilities in the model or data, leading to false negatives or incorrect classifications. Furthermore, machine learning models can inherit biases from the training data or algorithms used, resulting in unfair outcomes or discriminatory detection. This vulnerability can lead to evasion attacks, poisoning attacks, and erosion of trust in the model's credibility and reliability. As a result, addressing this limitation is crucial to ensure robust and fair cyber intrusion detection capabilities that can withstand sophisticated attacks and provide equal protection for all users and systems.

**Dependence on data representation and quality**

The effectiveness of machine learning models in cyber intrusion detection is heavily dependent on data representation and quality. Poor data quality, incomplete or inaccurate labeling, and inadequate feature representation can significantly compromise model performance. Additionally, the reliance on high-quality training data can lead to:

- Biased models trained on limited or skewed datasets
- Inadequate detection of unknown or novel threats
- High false positive rates due to noisy or irrelevant data
- Inability to generalize to new or changing environments
- Dependence on continuous data updates and maintenance
- Limited real-time detection capabilities

**Current trends**

**AI-driven threats:** AI is being leveraged to launch sophisticated attacks at an unprecedented scale, including AI-powered impersonation, phishing, malware, and ransomware.

**AI for threat detection:** AI is being utilized to detect and prevent cyber threats by identifying vulnerabilities and predicting attack patterns.[9]

**Generative AI:** Generative AI tools like ChatGPT are being used to craft highly personalized spear-phishing messages and deepfake voices to impersonate executives.[9][10]

**AI for incident response:** AI is improving incident response times and effectiveness by automating responses to common attacks and identifying potential security incidents.[9]

**AI for security information and event management:** AI is enhancing security information and event management systems by identifying potential security incidents and predicting attack patterns.[9]

**AI for cloud security:**AI is strengthening cloud security by identifying vulnerabilities and predicting attack patterns in cloud environments.[11]

**AI for identity and access management:**AI is optimizing identity and access management by identifying potential security incidents and predicting attack patterns.[11]

**AI for security orchestration, automation, and response:** AI is streamlining security orchestration, automation, and response by automating responses to common attacks and identifying potential security incidents.[11]

**The new era of AI-driven threats:** The hazards associated with AI are twofold: from outside corporate walls, companies confront an ongoing barrage of threats, including attacks powered by AI. The truth is that AI can help

almost any kind of threat that currently exists, which means that attacks can be conducted at a speed, sophistication, and scale never seen before. As for AI-driven cyberattacks, businesses confront an unknowable array of unknowns because the possibilities for the future are infinite.
But distinct attack patterns are starting to show. ThreatLabz offers insights into a variety of emerging threat categories in the 2024 AI Security Report, including**:**

- **AI impersonation:** Deepfakes created by AI, advanced social engineering techniques, false information, and more.
- **AI-Generated Phishing Campaigns:** End-to-end campaign generation using ChatGPT, demonstrated by ThreatLabz in a case study that created a phishing login page in 7 simple prompts.
- **AI-Driven Malware and Ransomware:** Threat actors leveraging AI automation across various stages of the attack chain**.**
- **AI-Generated Exploits:** Using ChatGPT to generate vulnerability exploits, as shown by ThreatLabz in creating proof-of-concepts for Log4j (CVE-2021-44228) and Apache HTTPS server path traversal (CVE-2021-41773).
- **Dark Chatbots:** The proliferation of dark web GPT models like FraudGPT and WormGPT, which lack security guardrails and pose significant threats.

**Impact of challenges and trends on organization:**
The challenges and trends in AI and ML for cybersecurity can have a profound impact on organizations. The increased risk of AI-powered cyber-attacks and data breaches can lead to significant financial losses and reputational damage. Moreover, developing and implementing effective AI-powered cybersecurity solutions requires substantial investment in resources and specialized expertise. The integration of AI systems also introduces new complexities and potential vulnerabilities, emphasizing the need for ongoing monitoring and updates to stay ahead of emerging threats. However, AI-powered cybersecurity solutions can also improve incident response times and reduce false positives, making them a crucial tool in the fight against cyber threats. Ultimately, organizations must adapt to the rapidly evolving AI and ML landscape to remain secure and resilient in the face of increasingly sophisticated cyber-attacks.

**Future directions for cyber security**
Significant progress in threat detection, incident response, and security analytics are expected in the field of cybersecurity in the future, thanks to the integration of artificial intelligence and machine learning. The migration of more data and apps to the cloud will make cloud security a priority. To stop assaults and data breaches, the increasing number of internet of things (IoT) devices will necessitate better security measures. Quantum-resistant encryption techniques and algorithms will be created in advance of quantum computing. Furthermore, zero trust architecture—which authenticates devices and users to guarantee safe access—will proliferate. To improve threat detection and incident response, solutions for extended detection and response (XDR) will also be incorporated. Moreover, cybersecurity education and awareness campaigns will take precedence, and laws pertaining to privacy and data security will tighten even more. Ultimately, cybersecurity will undergo a revolution with the development of autonomous security systems that can identify threats and take appropriate action without the need for human interaction.

## CONCLUSION

The landscape is changing as a result of the integration of machine learning (ML) and artificial intelligence (AI) in cybersecurity. This presents both opportunities and challenges. Advanced AI-powered cybersecurity solutions are necessary to identify and stop AI-powered threats, such as malware and phishing campaigns. More AI and ML integration, cloud security, IoT security, quantum computing, zero trust architecture, XDR solutions, and autonomous security systems are all part of the future of cybersecurity. Organisations need to invest in AI-powered

cybersecurity solutions, acquire specialised skills, and adjust to the quickly changing AI and ML landscape in order to remain ahead of emerging threats.

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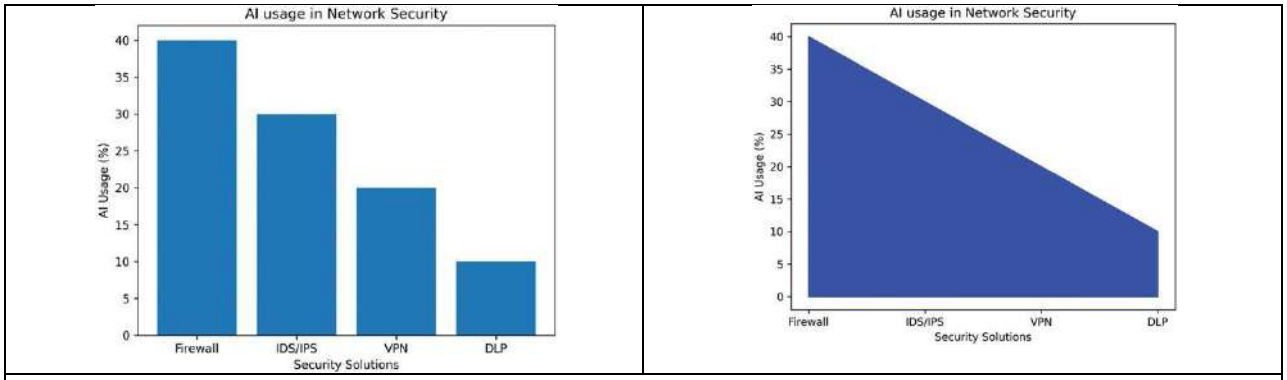


**Fig(1)& (2) Use AI ML in network security [2]**

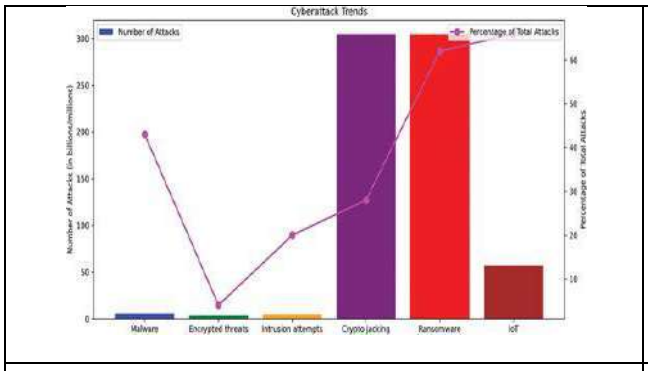


**Fig. 3: Present Cyber-attack trends [8]**

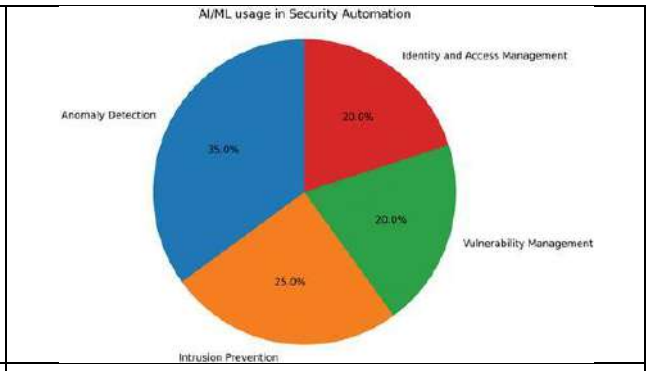


**Fig. 4:AI ML in security automation[8]**

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**RESEARCH ARTICLE**

# Enhancing Crystal Clarity : Exploring L-Threonine Diglycine Picrate (LTDGP) for Advanced Nonlinear Optics

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## ABSTRACT

The L-Threonine Diglycine Picrate (LTDGP) mono crystal was cultivated under normal temperature conditions using the Slow Evaporation Solution Technique (SEST). This technique involves allowing a saturated solution of LTDGP to slowly evaporate under controlled conditions, facilitating the gradual formation of well-defined crystals. Structural confirmation of the LTDGP crystalline material was conducted via single crystal X-ray diffraction, revealing a monoclinic system having the $P2_1/n$ space group. Powder X-ray diffraction was harnessed to discern the lattice structure of the LTDGP crystal. Vibrational modes and identification of chemical moieties in the formed LTDGP crystal were accomplished through Fourier Transform Infrared spectroscopy. The optical characteristics for the LTDGP crystal were gauged via UV- Visible-Near-Infrared spectroscopy. Electrical characteristics for the LTDGP crystal were explored by conducting dielectric experiments (constant and loss) as they vary with frequency at three different temperature. The LTDGP crystal has demonstrated an impressive laser damage threshold (LDT) of 1.9 GW/cm$^2$, underscoring its ideal suitability for laser-based applications. The Z-scan investigation distinctly revealed the nonlinear third-order susceptibility.

**Keywords:** Crystal growth, SXRD, PXRD, FTIR, UV-vis-NIR, Dielectric, LDT and Z-scan analysis.

# INTRODUCTION

Organic crystals, prized for their distinctive attributes, are extensively employed in various sectors. They serve as pivotal nonlinear optical materials, facilitating the control of light parameters such as intensity, phase, and polarization [1-2]. This functionality extends to optical switches, modulators, and frequency mixers, pivotal in telecommunications, signal processing, and optical data transmission. Additionally, organic co-crystals are gaining prominence in nonlinear optical (NLO) applications, leveraging their adaptable optical traits and heightened nonlinear response [3]. They hold promise in nonlinear frequency conversion, optical modulation, and advanced imaging techniques, driving progress in telecommunications, photonics, and ultrafast optics. Furthermore, organic co-crystals contribute to optical limiting systems, safeguarding optical apparatus from intense light pulses through nonlinear absorption mechanisms. These protective devices find utility in laser safety, military technology, and shielding delicate optical components [4]. Overall, organic co-crystals occupy a pivotal position in propelling nonlinear optical technologies forward, fostering innovation across photonics, telecommunications, imaging, and quantum optics realms. Diglycine picrate single crystals, comprised of diglycine and picric acid molecules organized in a precise lattice arrangement, have gained significant interest for their potential applications, particularly in explosives detection and nonlinear optics. A minimum of a few notable research papers have identified diglycine picrate as a compelling candidate within nonlinear optics (NLO), highlighting its unique optical properties and suitability for advanced optical devices and systems[5]. The combination of L-threonine with diglycine picrate single crystal holds promise for nonlinear optical (NLO) applications. This hybrid material can exhibit enhanced NLO properties compared to individual components, potentially enabling its use in devices such as optical modulators, frequency converters, or nonlinear imaging systems. Throughout this research, the organic L-Threonine diglycine picrate single crystal was created using the slow evaporation solution method for the maiden attempt. The resulting LTDGP crystal underwent characterization through S-XRD, P-XRD, UV-Visible-Near Infrared spectroscopy, LDT, and Z-scan analysis.

**Experimental section**

**Growth and synthesis procedure**

The creation of the LTDGP compound was achieved through the utilization of the slow evaporation solution technique. This involved dissolving L-Threonine and Diglycine Picrate at a 1:1 stoichiometric ratio. Initially, precise amounts of both substances were solvated separately in purified water, each in its own container. After complete dissolution, the solutions were gradually combined and stirred thoroughly for 8 hours under ambient conditions to guarantee full chemical reaction. The resulting solution displayed a yellow tint, indicating successful chemical conversion. Following this, the solution was left undisturbed to evaporate naturally at ambient conditions, culminating in the formation of yellow crystalline LTDGP salt. The growth of the well-formed L-Threonine diglycine picrate single crystal, measuring 12 mm × 10 mm × 7 mm, spanned 14 days. Figure.1 displays an image showcasing the LTDGP single crystal.

**Characterization techniques**

A comprehensive analysis was conducted on the LTDGP crystal using various techniques. For the structural examination, single crystal X-ray diffraction was executed utilizing a BRUKER AXS (kappaa APEXIII) x-ray diffractometer, employing graphite monochromated MoK$\alpha$ radiation with a wavelength of $\lambda$=0.71073Å. Additionally, powder x-ray diffraction investigation was executed employing a BRUKER D8 ADVANCE x-ray diffractometer. For vibrational characterization, assignments within the 400 to 4000 $cm^{-1}$ range were determined utilizing a BRUKER AXS FTIR Spectrometer. Mechanical properties were assessed through Vickers microhardness testing, conducted using a SHIMADZU-HMV-G21 model featuring a diamond tip. Further analysis included UV-visible analysis using a JASCO-V-730 Spectrometer, and dielectric analysis at three various temperature using an LCR Z meter (Agilent E 4980A) spanning a frequency spectrum from 50 Hz - 2 MHz. The exploration in to laser damage thresholds utilised a q-switched high- intensity Nd;YAG (1064 nm) laser from QUANTA RAY type. Finally,

the susceptibility in the LTDGP crystal was pinpointed through Z-scan analysis, opting for an uninterrupted beam laser (21 mW) at 632.8 nm wavelength.

## RESULTS AND DISCUSSION

**Structural determination by X-ray diffraction technique**

Single crystal X-ray diffraction unveils exact atomic structures through analysis of how X-rays interact with a single crystal sample. It delineates crystal symmetry, unit cell dimensions, and atomic positions with pinpoint accuracy. This method is pivotal in elucidating molecular structures in chemistry and materials science. Through assessment of diffraction patterns, it furnishes invaluable insights into the configuration and bonding of atoms within the crystal lattice. Single crystal XRD analysis is pivotal in comprehending the structure property correlations of organic crystals and guiding innovation in materials with desired characteristics [6].The lattice parameters for LTDGP include: a=5.086Ǻ, b= 11.802Ǻ, c=5.460 Ǻ ,$\alpha$=90$^0$;$\beta$=111.97$^0$;$\gamma$=90$^0$ along with its volume V=303.895(Ǻ)$^3$. Those data imply that the LTDGP crystal aligns with the monoclinic lattice, featuring a centrosymmetric space group designated as $P2_1/n$. Table.1 presents the specific data obtained from SXRD assessment for the LTDGP crystal.

**Powder XRD investigation**

Powder X-ray diffraction is a potent method employed for analyzing the crystallography within pulverized samples, revealing information about their atomic and molecular arrangement [7]. The measurements were conducted over a 2θ degree range from 10$^0$-70$^0$ at ambient temperature. From the derived graph, the highest intensity occurred at an angle of 29.889°. The existence of prominent, clear-cut peaks suggests the high-quality structured crystal lattice in the LTDGP crystal. The PXRD spectra of LTDGP appear in figure 2, with the powder XRD data are furnished within table 2.

**Fourier Transform Infrared Spectroscopy**

FTIR spectroscopy detects how a sample absorbs or emits infrared light. FTIR spectroscopy utilizes infrared radiation to investigate molecular vibrations, offering intricate details about the molecular bonds and functional moieties present in a specimen. Through analysis of absorption patterns across diverse infrared wavelengths, FTIR effectively discerns between various substances and reveals their molecular structures [8]. Figure.3 displays the FTIR spectrum of LTDGP, acquired via the KBr pellet method, spanning the 500 to 4000 cm$^{-1}$ range. Notably, a broad spectrum is observed spanning the 1600-3200 cm$^{-1}$ range, showcased in Figure 3. The absorption bands and their modes are detailed in Table 3. These assignments offer insights into the functional groups within the substance and the corresponding vibrational patterns observed within the FTIR spectrum [9]. Infrared spectral peaks were recorded for various compounds, and their respective wave numbers and assignments are as follows: A peak at 3156.24 cm$^{-1}$ signifies O-H stretching typical of carboxylic acids, while a peak noted at 2887.77 cm$^{-1}$ indicates N-H stretching characteristic of amine salts. Additionally, another O-H stretching peak detected at 2601.82 cm$^{-1}$ suggests the existence of carboxylic acid functionality. The N-H bending vibration detected around 1582.24 cm-1 suggests an amine group, whereas the peak noticed around 1497.16 cm-1 denotes the N-O stretching vibration characteristic of nitro groups. Furthermore, the S=O stretching vibration at approximately 1403.71 cm$^{-1}$ suggests the existence of a sulfate moiety, whereas the O-H bending vibration near 1327.37 cm$^{-1}$ is linked to phenol groups. The C-O stretching vibration detected near 1112.43 cm$^{-1}$ implies the existence of an aliphatic ether, and the S=O stretching vibration discerned around 1033.14 cm$^{-1}$ is indicative of sulfoxides. Additional peaks include C=C bending vibrations at 893.62 cm$^{-1}$ for alkenes and at 691.42 cm$^{-1}$ for disubstituted (cis) alkenes. Finally, the C-Br stretching vibration noted around 604.89 cm$^{-1}$designates presence of halo compounds.

**Microhardness Testing**

Valuable insights into the mechanical properties of crystals at small scales are gleaned through microhardness testing. This method measures the resistance of a material to indentation or penetration, providing a crucial

understanding of its hardness. Such insights are essential for assessing the material's suitability for specific applications [10].

Figure 4(a) presents a plot showing the logarithmic interdependence of the logarithm of P and the logarithm of d. The measured value of n (B=0.26341) on the graph confirms that the LADGP crystal falls within the hard category [11]. In Figure 4(b), a graphical representation demonstrates the correlation of the hardness number 'Hv' with exerted load 'P'. Examining the plot, it's apparent that with the increase in weight from 25g to 50g, the hardness value (Hv) exhibits a linear increase with the load, whereas beyond 50g, Hv rises steadily. The hardness value obtained from the grown LTDGP crystal indicates a need for higher stress to induce micro damages, affirming its superior crystalline perfection [12]. This quality makes the crystal highly suitable for device fabrication purposes.

**UV-vis NIR Evaluation**

Ultraviolet-Visible-Near-Infrared spectroscopy is a robust analytical method employed for examining the optical characteristics of crystals over a broad spectrum, typically encompassing ultraviolet (UV) to near-infrared (NIR) wavelengths. UV-Vis-NIR spectroscopy provides insights into crystal optical properties, aiding material identification, bandgap determination, and defect analysis [13]. In figure 5(a) showcase the optical transmittance spectra for the LTDGP single crystal. The LTDGP crystal demonstrates an impressive transmittance rate of 85%, spanning a wide range from 500 to 1100 nm, which presents significant advantages across various optical applications. Across the UV-visible NIR spectrum, the LTDGP crystal displays notable transmission and minimal absorption. Figure 5(b) highlights the graph of ($\alpha$hv) against (hv), providing clarification on the energy band gap spectrum. Extrapolating from the linear segment of the curve yields an estimated optical band gap at 4.9 eV for the LTDGP crystal. Furthermore, Figure 5(c) exemplifies the absorption spectrum of the LTDGP single crystal, highlighting the lower cut-off wavelengths observed at 210 nm. These absorption characteristics are attributed to specific transitions, particularly the n-$\sigma^*$ transition within the LTDGP single crystal [14]. The optical characteristics of the LTDGP crystal are evident from the distinct increase in optical conductivity at 250 nanometers, as exposed in Figure 5(d). Figure 5(e) portrays the refractive index changes with wavelength, revealing a lower refractive index of 0.023 within the visible spectrum of the LTDGP crystal. In tandem with the increase in wavelength of light, there is a corresponding decrease in the refractive index for the material. The extinction coefficient in the LTDGP crystal changes with varying wavelengths, as depicted in Figure 5(f). It is directly linked with the absorption coefficient in the LTDGP crystal. When considering wavelength within the visible region, the extinction coefficient reaches its minimum. Therefore, this suggests that the absorption of radiation by the LTDGP crystal will also be minimal within the visible spectrum. Therefore, the LTDGP crystal's broader band gap, reduced refractive index, and strong transmission across the spectrum render it highly suitable for advanced optoelectronic applications.

**Dielectric study**

Dielectric studies delve into the electrical behavior of materials, particularly focusing on how they respond to electric fields. Investigating factors like dielectric constant, loss tangent, and frequency dependence reveals insights crucial for applications ranging from telecommunications to materials science. Understanding the intricate interplay between crystal structure and electrical properties enables the development of advanced technologies with tailored functionalities. Knowledge of dielectric properties in crystals finds applications in diverse fields such as semiconductor devices, piezoelectric materials, optical devices, sensors, and energy storage devices like capacitors. [15]. The dielectric analysis conducted on LTDGP single crystal revealed intriguing insights into its properties across a diverse frequency span, ranging from 1Hz-1MHz, under ambient conditions. Figures 6(a) and (b) serve as visual representations of the correlation between the dielectric permittivity and dissipation in the LTDGP crystal. Figure 6(a) highlights notable dielectric constant values observed at reduced frequencies in the graph. This phenomenon suggests the existence of diverse polarizations within the crystal structure, including ionic, electronic, space charge polarization, and orientation [16]. These variations in polarization mechanisms play a role in the observed dielectric constant values, particularly pronounced during lower frequency ranges. On the other hand, Figure 6(b) highlights the minimal dielectric loss exhibited by the LTDGP single crystal across the frequency spectrum. This low level of dielectric loss underscores the crystal's exceptional optical quality, characterized by minimal defects. Such high

optical quality is advantageous for applications in nonlinear optics (NLO), where pristine crystal properties are crucial for efficient nonlinear optical processes [17]. In Figure 6(c), It is palpable that as the frequency escalates, the value of $\sigma_{ac}$ also rises in the LADGP single crystal. Table 4.presents the activation energy established in LTDGP. Overall, the findings from the dielectric analysis emphasize the promising potential of LTDGP single crystal for various applications, particularly in nonlinear optics, due to its favorable dielectric properties and enhanced optical quality.

**Laser Damaged Threshold study**

Laser damage threshold studies on crystals are quite common within the realm of optics and photonics. The laser damage threshold signifies the maximum intensity of a laser pulse that a material can withstand before sustaining damage. The threshold may fluctuate based on aspects like the crystal type, its purity, surface condition, and laser beam attributes [18]. L-Threonine diglycine picrate (LTDGP) is a specific type of organic nonlinear optical crystal. The LDT assessment for the LTDGP crystal included exposing it to a high-energy Nd:YAG laser-[1064 nm] utilizing a Q-switched setup. A 6 ns pulse duration was utilized, with the laser exposure set to 1064 nm directed towards the LTDGP specimen at a frequency 10Hz. The incoming laser light concentrated with a focal length at 10 cm. The pulse output of the laser light was gauged employing a power meter, and energy density was determined utilizing the prescribed equation,

$$\text{Power density, } P_d = E/\tau\pi r^2$$

In this formula, 'E' denotes the energy flux measured in millijoules (mJ), 'τ' symbolizes the pulse duration at nanoseconds (ns), and 'r' signifies the target radius in millimeters (mm). Remarkably, the LADGP crystal exhibits an impressive laser damage threshold of 1.9 GW/cm², surpassing that of KDP (0.20 GW/cm²) and Urea (1.50 GW/cm²). The exceptional optical damage tolerance of the LTDGP crystal underscores its characteristics. The investigation into the LTDGP crystal contributes to the progress of nonlinear optical devices and systems, which play pivotal roles in various domains such as telecommunications, laser spectroscopy, and laser-based material processing.

**Z-Scan Investigation**

Z-scan modality is a formidable approach that enables the exploration of nonlinear optical traits in materials. The LTDGP crystal underwent analysis via the Z-scan approach, which is serves to elucidate its third-order nonlinear optical characteristics, specifically the nonlinear refractive index (n2), absorption coefficient (β), and third-order susceptibility ($\chi^{(3)}$). A Helium-Neon laser source emitting at 632.8 nm boasting a power output measuring 21 mW was utilized. The LTDGP crystal was positioned vertically on a holder, featuring a thickness (L) of 1 mm, ensuring the laser could traverse the material without obstruction. The automated stepper motor system regulates the positioning of the crystal holder precisely to the focal point, with the photodetector capturing the respective intensity of transmittance [20-21]. The open and closed window Z-scan diagrams are visualized in Figures 8(a) & 8(b), in their respective positions. The n2 and β for the LTDGP crystal were derived based on the open and closed aperture data for each. The crystal induced a peak-to-valley variation at the closed aperture, indicating the presence of the self-defocusing effect, as illustrated in Figure 8(a). On the contrary, in the open aperture configuration, the LTDGP crystal showcased its bottommost transmittance point near the focal point (Z0), unveiling reverse saturable absorption [22], as delineated in Figure 8(b). The evaluation of the third-order nonlinear refractive index 'n2' registered $8.7970\times10^{-12}$ m²/W for the LTDGP crystal under closed aperture conditions. In the open aperture setup, the computed nonlinear absorptivity 'β' for the LTDGP crystal is 9.9580×10-5 m/W. Based on the attained values, the actual 'Re($\chi^{(3)}$)' and imaginary 'Im($\chi^{(3)}$)' susceptibilities of the LTDGP crystal are established as $7.2308\times10^{-10}$ and $4.1238\times10^{-8}$ (esu),each. The actual and imaginary components in the third-order nonlinear - susceptibility in an LTDGP crystal are captured by the equation provided in reference [23],

$$|\chi^{(3)}|=(\mathrm{Re}[\chi^{(3)}])^2+(\mathrm{Im}[\chi^{(3)}])^2$$

The equation is utilized to derive the magnitude of the third-order nonlinear susceptibility ($|\chi^{(3)}|$) from its actual ($\mathrm{Re}[\chi^{(3)}]$) and imaginary ($\mathrm{Im}[\chi^{(3)}]$) parts. The $\chi^{(3)}$ value for LTDGP was computed as $4.12382\times10^{-8}$ esu. The

susceptibility value acquired is superior to that of alternative materials in nonlinear optics [24-27].The inherent self-defocusing property of the LTDGP single crystal presents numerous opportunities for various functions, like holographic storage, optical tweezers, and nonlinear wavefront shaping[28].

## CONCLUSION

A superior monolithic crystal of LTDGP was produced employing the slow evaporation solution method, resulting in a clear yellow crystal measuring 12 x 10 x 7 $mm^3$. Analysis using single crystal X-ray diffraction exposed the crystal system of LTDGP is monoclinic with centrosymmetric space group $P2_1/n$. The LTDGP crystal's purity was established through powder X-ray diffraction. The vibrational characteristics and functional moieties of LTDGP crystals were examined via FTIR spectroscopy. Vicker microhardness test was used to evaluate its hardness properties. Spectroscopic analysis in the UV-Visible-Near infrared range showed that the LTDGP crystal possesses a transmittance of 85%, with cut-off wavelengths at 210 nm, highlighting its potential for Nonlinear Optical (NLO) applications. The LTDGP crystal demonstrated excellent optical quality and minimal dielectric loss at high frequencies, as indicated by its dielectric response. The Laser Damage Threshold (LDT) of LTDGP was assessed at 1.9 $GW/cm^2$, highlighting its suitability for applications involving lasers. Additionally, the LTDGP crystal's susceptibility ($\chi^{(3)}$) value was measured at $4.12382\times10^{-8}$ esu, confirming its practical utility in fields such as optical communication and the development of photonic devices.

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**Table 1.SXRD data for LTDGP single crystal**

| Sample | Cell parameters | Volume (Å)$^3$ | Z |
|---|---|---|---|
| L-Threonine Diglycine Picrate single crystal | a= 5.086Å<br>b= 11.802Å<br>c= 5.460 Å<br>$\alpha$=90$^0$; β=111.97$^0$<br>γ=90$^0$ | 303.895 | 4 |

**Table 2.PXRD details about LTDGP single crystal**

| Pos.[◦2Th.] | Height[cts] | FWHM[◦2Th.] | d spacing[Å] | Rel.int.[%] | h k l |
|---|---|---|---|---|---|
| 14.758 | 244477.5 | 0.094 | 5.99776 | 32.8 | 1 0 1 |
| 19.977 | 87.0186 | 0.095 | 4.44098 | 0.1 | 1 1 1 |
| 24.138 | 266.803 | 0.147 | 3.68413 | 0.4 | 2 1 0 |
| 29.281 | 4972.44 | 0.128 | 3.04713 | 6.7 | 2 1 1 |
| 29.886 | 74637.5 | 0.131 | 2.98726 | 100 | 1 1 2 |
| 56.891 | 1177.09 | 0.152 | 1.61718 | 1.6 | 2 1 1 |

**Table 3: FTIR spectral assignments of LTDGP**

| Wave Number (cm$^{-1}$) | Assignment modes | Compound |
|---|---|---|
| 3156.24 | O-H stretching | Carboxylic acid |
| 2887.77 | N-H stretching | Amine salt |
| 2601.82 | O-H stretching | Carboxylic acid |
| 1582.24 | N-H bending | Amine |
| 1497.16 | N-O stretching | Nitro group |
| 1403.71 | S=O stretching | Sulfate |
| 1327.37 | O-H bending | Phenol |
| 1112.43 | C-O stretching | Aliphatic ether |
| 1033.14 | S=O stretching | Sulfoxide |
| 893.62 | C=C bending | Alkene |
| 691.42 | C=C bending | Disubstituted(cis) |
| 604.89 | C-Br stretching | Halo compound |

**Table 4.Activation energy in LTDGP**

| Frequency(Hz) | Ea/K | in eV |
|---|---|---|
| 100 | 4.15314 | 0.000357876 |
| 1099 | 3.91516 | 0.000337369 |
| 10095 | 3.45853 | 0.000298022 |
| 50075 | 3.12805 | 0.000269544 |
| 100050 | 3.02736 | 0.000260868 |
| 200000 | 2.56505 | 0.00022103 |

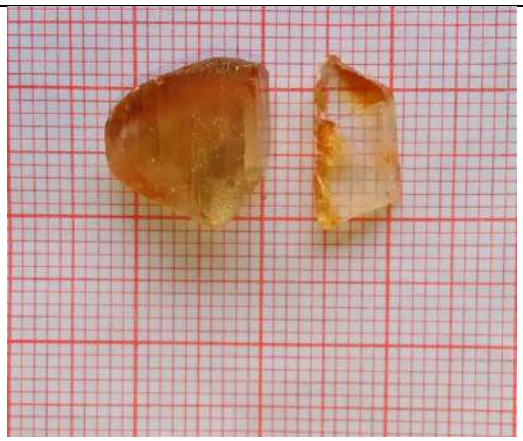


**Figure.1 As grown LTDGP single crystal**

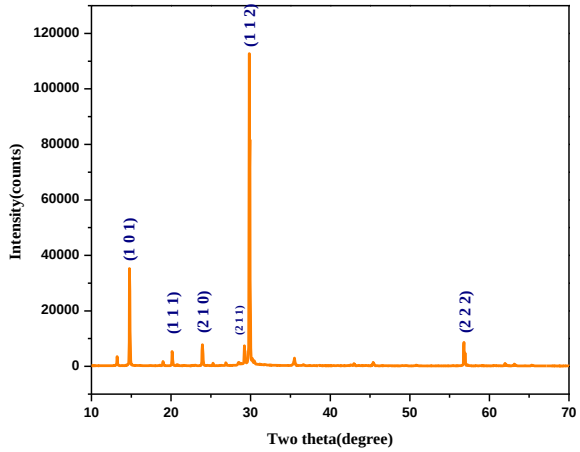



**Figure2.XRD pattern of LTDGP with hkl indices**

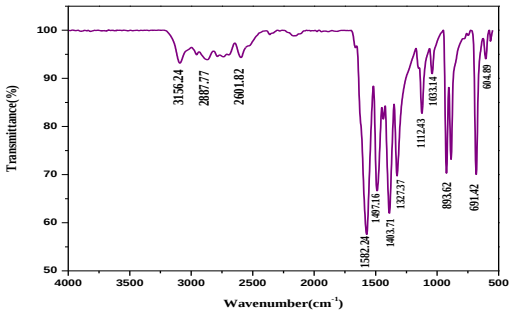



**Figure3. FTIR spectra of LTDGP**

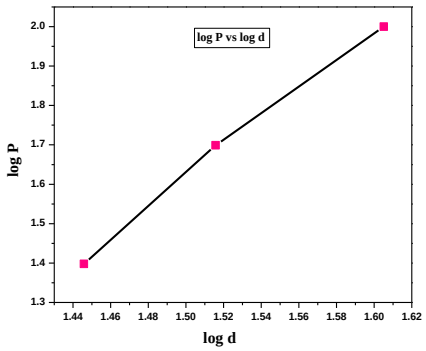



**Fig.4a) variation of log P against log d**

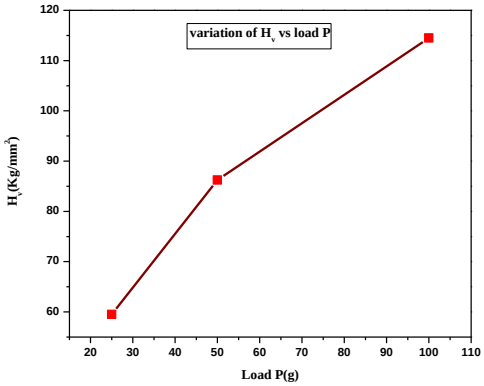
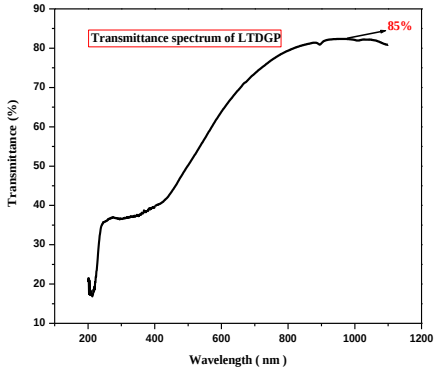
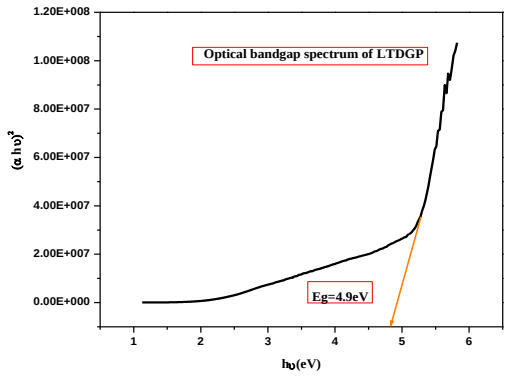


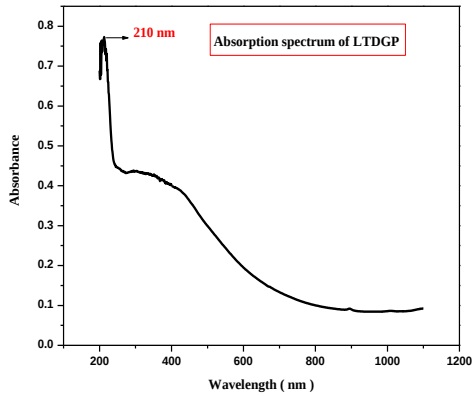

**Fig.4b) variation of Hv against load P**



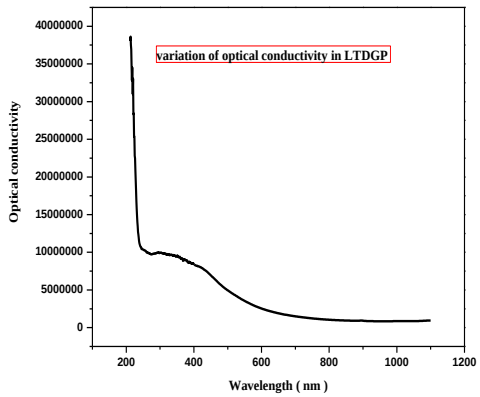

**5a) Transmittance vs wavelength**



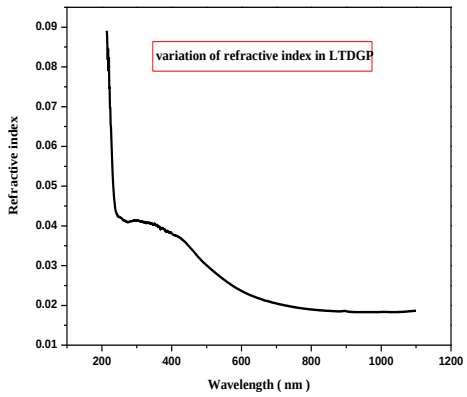

**5b) Tauc plot of LTDGP**



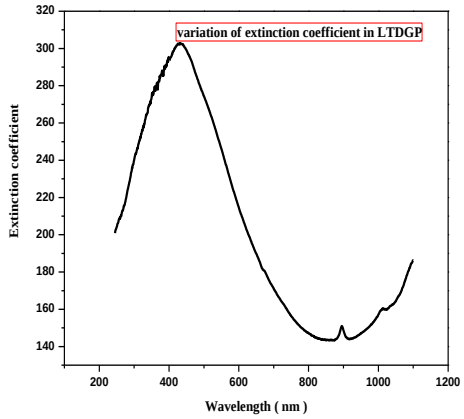

**5c) Absorbance vs wavelength**




**5d) Optical conductivity vs wavelength**




**5e) Refractive index with wavlength**




**5f) Extinction coefficient with wavelength**

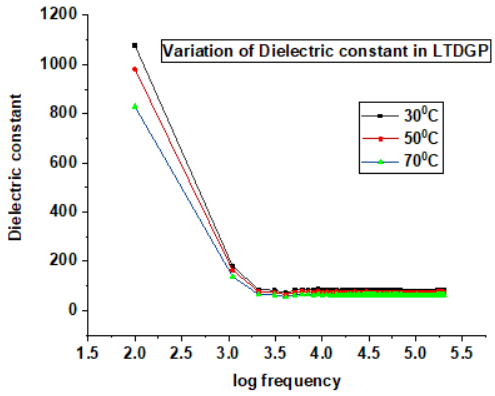



**Fig. 6a) variation of dielectric constant in LTDGP**

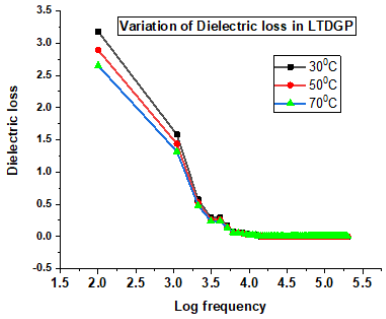



**Fig.6b) variation of dielectric loss in LTDGP**

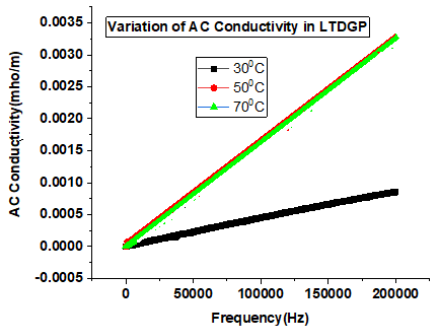



**Fig. 6c) variation of AC conductivity in LTDGP**

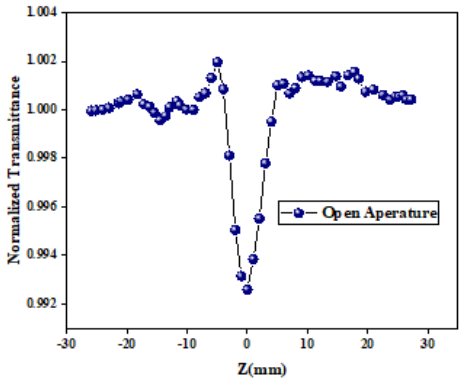



**Fig.7(a)visualizes open aperture curve of LTDGP**

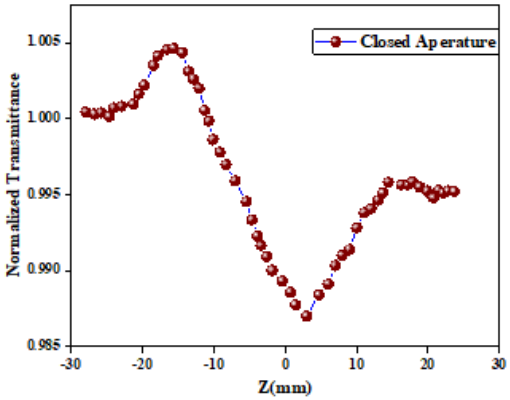



**Fig.7(b) visualizes closed aperture curve of LTDGP**

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**RESEARCH ARTICLE**

# Comparative Analysis of Risk Management Practices in Public and Private Sector Banks

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**ABSTRACT**

This theoretical research paper explores the "Comparative Analysis of Risk Management Practices in Public and Private Sector Banks," focusing on how these institutions address various types of risks, including credit, market, operational, and regulatory risks. By conducting a comprehensive review of existing literature, the paper identifies and contrasts the risk management strategies employed by public sector banks, which are largely government-owned and driven by stability and compliance, with those used by private sector banks, which are motivated by profitability and competition. The paper examines how ownership structure, regulatory environments, and organizational culture shape the risk management practices in each sector. Public sector banks tend to follow more conservative, policy-driven risk management approaches, while private sector banks often adopt innovative, dynamic strategies to respond to changing market conditions. The theoretical comparison highlights the strengths and weaknesses of both approaches, offering insights into potential improvements for risk management practices. The study concludes that a balanced, cross-sectoral learning approach could enhance risk management frameworks in both public and private sector banks, ensuring greater financial stability and resilience in an evolving economic landscape.

**Keywords:** Risk Management, Public Sector Banks, Private Sector Banks, Credit Risk, Organizational Culture, Financial Stability.

## INTRODUCTION

The banking sector plays a pivotal role in the stability and growth of any economy, acting as the backbone for financial intermediation. Banks facilitate the efficient allocation of resources, support trade and commerce, and provide a secure environment for savings and investments. However, this vital role exposes banks to a variety of

risks that can have significant consequences if not properly managed. Effective risk management, therefore, becomes a cornerstone of banking operations, ensuring the stability and continuity of financial institutions. In this context, the risk management practices adopted by banks, particularly in public and private sectors, have garnered considerable attention from policymakers, regulators, and researchers alike.

Public and private sector banks operate under different ownership structures, regulatory frameworks, and organizational cultures, which directly influence their approach to managing risks. Public sector banks, often characterized by government ownership and a focus on social objectives, are subject to strict regulatory oversight and may prioritize stability and compliance over profitability. In contrast, private sector banks, driven by market competition and profit motives, tend to adopt more dynamic and innovative risk management practices. These differences raise important questions about the effectiveness of risk management strategies in the two sectors and their implications for the overall stability of the banking system. The concept of risk management in banking encompasses a wide range of activities aimed at identifying, assessing, mitigating, and monitoring risks. These risks can be broadly categorized into credit risk, market risk, operational risk, liquidity risk, and reputational risk, among others. Credit risk, for instance, refers to the potential for loss arising from a borrower's failure to meet their obligations. Market risk involves the possibility of losses due to changes in market conditions, such as interest rates, exchange rates, or stock prices. Operational risk stems from failures in internal processes, systems, or external events, while liquidity risk is associated with a bank's inability to meet its financial obligations when they fall due. Reputational risk, although more abstract, can have severe consequences if a bank's credibility is compromised.

In public sector banks, risk management practices are heavily influenced by government policies and the need to balance profitability with social objectives. These banks often operate in less competitive environments and may have access to government support in times of financial distress. However, this reliance on government backing can also lead to moral hazard, where banks may take on excessive risks with the expectation of being bailed out. Moreover, the bureaucratic nature of public sector banks can result in slower decision-making processes, potentially hindering the timely identification and mitigation of risks.

Private sector banks, on the other hand, operate in a highly competitive environment where the pursuit of profitability and shareholder value drives their risk management strategies. These banks tend to be more agile and responsive to changes in market conditions, adopting advanced risk management tools and techniques. The focus on profitability, however, can sometimes lead to higher risk-taking behavior, particularly in areas such as lending and investments. Regulatory frameworks for private sector banks, while robust, may not always keep pace with the rapid evolution of financial markets and products, creating potential gaps in risk oversight. The theoretical comparison of risk management practices between public and private sector banks offers valuable insights into the strengths and weaknesses of each approach. For instance, while public sector banks may benefit from greater regulatory oversight and stability, they may also face challenges related to efficiency and innovation. Private sector banks, in contrast, may excel in adopting cutting-edge risk management techniques but must carefully balance risk-taking with the need for regulatory compliance and financial stability.

This article aims to explore these differences in risk management practices from a theoretical perspective, drawing on existing literature and frameworks to compare the approaches of public and private sector banks. By examining the underlying principles that guide risk management in these two sectors, the article seeks to contribute to a deeper understanding of how ownership structure, regulatory environment, and organizational culture influence risk management outcomes. Furthermore, the article will discuss the implications of these theoretical insights for banking policy and practice, offering recommendations for improving risk management across the banking sector. In conclusion, the comparative analysis of risk management practices in public and private sector banks is a critical area of study, given the central role that banks play in the financial system. Understanding the theoretical foundations of these practices can help identify areas for improvement and enhance the resilience of the banking sector in the face of evolving risks. As the financial landscape continues to change, particularly with the rise of digital banking and

fintech innovations, it becomes increasingly important for both public and private sector banks to adapt their risk management strategies to ensure their long-term sustainability and success.

## LITERATURE REVIEW

Sinha and Gupta (2016) investigated risk management practices in Indian banks, focusing on the integration of technology. They highlighted that private sector banks were early adopters of risk management software and digital platforms, enabling real-time assessments and mitigation strategies. Public sector banks, on the other hand, were slower in adopting technology-driven solutions, which contributed to the superior risk management capabilities observed in private banks. Singh and Reddy (2016) analyzed the impact of regulatory compliance on the risk management frameworks of public and private sector banks. Their findings indicated that while both sectors adhered to regulatory guidelines, private banks implemented more advanced risk control measures compared to public sector banks, which typically met only the minimum regulatory requirements. Mahapatra and Mohanty (2017) focused on stress testing and capital adequacy practices, finding that private sector banks implement stress testing mechanisms more rigorously than their public counterparts. They argued that public sector banks lagged due to their rigid hierarchical structures, affecting their ability to react swiftly to global financial changes.

Jain and Kaur (2017) conducted a comparative study on market risk management in public and private sector banks. Private sector banks were found to have more advanced market risk monitoring tools, including real-time market data analytics. Public banks, in contrast, lacked technological infrastructure, making them slower to respond to market fluctuations. Garg and Shukla (2017) explored the role of capital adequacy in managing financial risks across public and private sector banks. They found that private sector banks maintained higher capital adequacy ratios, providing a stronger financial cushion during economic uncertainty. Public sector banks, however, operated with lower capital reserves, making them more vulnerable during financial crises. Kumar and Sharma (2018) studied governance structures behind risk management in public and private sector banks. Their research showed that private banks had more dynamic governance frameworks, with an emphasis on early risk identification and mitigation, while public sector banks were slower to respond to emerging risks due to bureaucratic constraints.

Sharma and Joshi (2018) assessed the role of regulatory compliance in shaping risk management strategies in both sectors. They found that private sector banks often went beyond regulatory compliance by implementing additional risk control measures, while public sector banks focused more on adhering to minimum requirements. Verma and Singh (2018) examined credit risk management practices in public and private banks. Their research revealed that private banks utilized sophisticated credit scoring models and advanced data analytics, enabling them to mitigate credit risks more effectively than public sector banks, which relied more on manual credit assessments. Shukla and Tandon (2018) studied credit default risk management in public and private sector banks. They found that private banks were more effective in managing credit defaults, largely due to their use of advanced credit monitoring systems, while public sector banks faced higher default rates.

Choudhury and Gupta (2019) provided an in-depth look into risk management practices in Indian public and private sector banks. They found that private banks were more proactive in adopting advanced credit scoring models and risk-based pricing mechanisms, while public sector banks were slower in implementing these strategies due to governmental constraints. Mishra and Roy (2019) examined governance and risk culture in banks. Private sector banks cultivated a stronger risk-aware culture, with employees encouraged to report risks at an early stage, while public sector banks had a more reactive approach. The authors argued that fostering a proactive risk culture in public sector banks could improve their ability to manage emerging risks. Raj and Natarajan (2019) explored how global financial instability influenced risk management practices. Their study showed that private banks adjusted their risk management frameworks more rapidly in response to global financial trends, whereas public sector banks maintained a more conservative approach, making them less adaptable.

Bhatia and Khosla (2019) investigated the use of risk-based auditing in public and private sector banks. Private banks employed more sophisticated risk-based audit systems, allowing them to identify potential risks at early stages. Public sector banks relied more on traditional audit systems, which were less effective in identifying emerging risks.

Ahmad and Bhatti (2020) conducted a comparative analysis of risk management practices in public and private sector banks post-global financial crisis. They found that public sector banks were more focused on regulatory compliance, while private banks adopted innovative and flexible risk management approaches, particularly in terms of technology and efficiency. Ravi and Suresh (2020) explored the role of human capital in risk management, highlighting that private sector banks invested more in employee training and development related to risk management, whereas public sector banks were slower to update their employees' knowledge of new tools and techniques. Patel and Mehta (2020) examined liquidity risk management practices. Private banks were found to maintain more stringent liquidity buffers, ensuring better preparedness for liquidity crises, while public banks operated with thinner margins, leaving them more vulnerable during financial distress.

Arora and Kumar (2021) compared operational risk management strategies in public and private banks. They found that private sector banks employed more dynamic internal control mechanisms to monitor operational risks, while public sector banks were slower in integrating automated systems for risk management.

Singh and Reddy (2021) explored how regulatory reforms impacted risk management practices. Their study revealed that private sector banks were quicker to adopt new regulatory measures, such as the Basel III guidelines, while public sector banks faced delays due to procedural issues, affecting their ability to improve risk management frameworks.

**Objectives:**

1. To Identify Key Risk Management Practices in Public and Private Sector Banks.
2. To Theorize the Impact of Ownership Structure on Risk Management Approaches.
3. To Compare Regulatory Influences on Risk Management in Public and Private Sector Banks.
4. To Explore Organizational Culture's Role in Shaping Risk Management Strategies.
5. To Theorize the Relationship Between Risk Management and Bank Performance.
6. To Discuss Theoretical Implications for Future Banking Policies and Risk Management Practices.

## RESEARCH METHODOLOGY

The research methodology for this theoretical paper involves a qualitative approach, primarily focusing on an extensive literature review to compare and contrast risk management practices in public and private sector banks. Secondary data from peer-reviewed journals, books, industry reports, and regulatory documents were meticulously examined to gain a comprehensive understanding of the theoretical frameworks and strategies employed in both sectors. The selection of literature was guided by its relevance to the subject, particularly studies that address risk identification, assessment, mitigation, and monitoring within the banking industry. This paper employs established theoretical frameworks, such as agency theory and stakeholder theory, to analyze the underlying factors that influence risk management practices in public and private sector banks. Through a systematic comparative analysis, the paper explores key aspects like credit risk management, market risk management, and regulatory compliance. The synthesis of findings from this analysis allows for the development of a cohesive theoretical understanding of the differences and similarities in risk management approaches between the two sectors, providing insights into potential areas for improvement and further research.

## FINDINGS

The theoretical research reveals several key findings regarding the differences in risk management practices between public and private sector banks:

1. Influence of Ownership Structure:
- Public sector banks, being government-owned, tend to prioritize stability and compliance over profitability. Their risk management practices are heavily influenced by government policies and social objectives, leading to a more conservative approach.
- Private sector banks, driven by profit motives and market competition, adopt more dynamic and innovative risk management practices. Their approach is often more flexible and responsive to changes in market conditions, but this can also lead to higher risk-taking behavior.
2. Regulatory Environment:
- Public sector banks operate under strict regulatory oversight, which ensures adherence to risk management standards but can also limit their ability to innovate and adapt quickly.
- Private sector banks, while also subject to regulatory requirements, often have more leeway to experiment with new risk management tools and strategies. However, this can sometimes result in gaps in regulatory compliance, especially in rapidly evolving financial markets.
3. Organizational Culture:
- The bureaucratic nature of public sector banks can slow down decision-making processes, potentially hindering the timely identification and mitigation of risks.
- In contrast, private sector banks typically have a more agile and performance-driven culture, which encourages quicker responses to emerging risks. However, this can also increase the likelihood of aggressive risk-taking in pursuit of higher returns.
4. Theoretical Implications:
- The differences in risk management practices between public and private sector banks can be explained through theoretical lenses such as agency theory and stakeholder theory. Public sector banks prioritize the interests of the government and the public, leading to a more cautious approach, while private sector banks focus on maximizing shareholder value, resulting in more risk-oriented strategies.

## CONCLUSION

The comparative analysis of risk management practices in public and private sector banks highlights the influence of ownership structure, regulatory environment, and organizational culture on their approaches to managing risks. Public sector banks, with their government backing and focus on social objectives, tend to adopt more conservative risk management practices, prioritizing stability and compliance. While this approach minimizes risk, it can also hinder innovation and responsiveness. On the other hand, private sector banks, driven by market competition and profitability, embrace more innovative and dynamic risk management strategies. Their ability to adapt quickly to changing market conditions gives them an edge in certain areas, but it also exposes them to higher risks, particularly when regulatory oversight is insufficient.

The findings suggest that while both public and private sector banks have their strengths and weaknesses in risk management, there is potential for cross-learning between the two sectors. Public sector banks can benefit from adopting more innovative risk management practices from the private sector, while private sector banks can strengthen their regulatory compliance and risk governance by emulating the more structured approaches of public sector banks. In conclusion, the theoretical insights from this research underscore the importance of a balanced approach to risk management in banking. As the financial landscape continues to evolve, both public and private sector banks must adapt their risk management practices to ensure long-term stability and resilience. Future research could explore the practical implications of these theoretical findings, particularly in the context of emerging risks such as cybersecurity threats and climate-related financial risks.

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**RESEARCH ARTICLE**

# Defending Your Trademark Rights : Protection Strategies

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**ABSTRACT**

The main purpose of a trademark is to identify the source of a product and distinguish it from goods that come from other sources. To ensure your rights and safeguard your intellectual property, you must register a trademark. This article provides an overview of trademarks, their protection, role, and infringement. Additionally, it covers the trademark system and laws of trademarks in India. The trademark owner or any licensees must authorize trademark infringement, which is a violation of their exclusive rights. India's trade mark laws were passed in 1999 and are currently in force. Mumbai is home to the Department of Trademarks' main office. Trademarks are actives having a high value for any company. The impact of trade mark fluctuations on a company's market journey is significant.

**Keywords**: Trademark, infringement, exclusive rights, trademark laws, authorization.

## INTRODUCTION

A trademark is a term that encompasses any word, symbol, or design that is used to identify the products or services of a particular party from those of another. Since the mark owner is the only one with this authority, competitors are not permitted to exploit the goodwill associated with a trademark through unfair trade activities, such as product mislabelling, fraudulent advertising, or counterfeiting. To ensure a certain level of regularity and, in some cases, quality in purchased goods, the legislation expressly prohibits the use of markings that increase the likelihood of confusion in the marketplace, cause an error, or deceive regarding the source of the items to which they are linked. Here's another way that consumers can benefit from trademark protection. Trademark protection can benefit consumers as well, since the law specifically prohibits the use of marks that are likely to mislead, confuse, or confuse customers about the origins of the products to which they are tied. This aids in ensuring a specific degree of consistency and, occasionally, quality in the purchased goods.(1) Selecting the right trademark and designing a logo or slogan are a company's two main and most important tasks. Whenever possible, steer clear of phrases that are

suggestive, descriptive, or generic. It is believed that the most effective type of trademarks is fancy names. A compound term consisting of two words with dictionary definitions that isn't being used by another corporation might be the fancy trademark.(2)

**THE ROLE OF TRADEMARKS**

The primary objective of trademark protection was to stop rivals from misrepresenting the products of one producer as their own. Their secondary objective is intimately related to trademark protection against unfair competition. Helping customers find high-quality products is the aim of this second job. By allowing customers to track a product's origins, trademarks assist consumers in making judgments during the purchasing process. Customers have the option to return merchandise bearing a certain trademark or decide not to buy it at all.There is a claim that trademarks need legal protection in order to serve as a buffer between consumers and market instability. Trademarks allow consumers to confirm the quality of a product based on their own experiences. Consequently, rather of offering a quality guarantee, trademarks offer a consistency guarantee. The original primary function of trademarks, which was to indicate origin, has changed in the modern day due to brand proliferation.

In industrialized markets, trademarks are no longer always a trustworthy indicator of provenance. Nonetheless, as trademarks signify the owner's good intentions, they are legally protected.Customers are delighted and goodwill is fostered by the products' exceptional quality and/or the trademark owner's marketing initiatives. It has been questioned how valuable trademarks are for their secondary purpose of quality identification. The following points were highlighted in a report published by the Economic Council of Canada in this respect.One way to measure market dominance is through a time-limited monopoly, which in the case of trademarks is obtained by boosting brand loyalty. Even though their potential economic results are similar, their fundamental assumptions are very different. Trademarks are therefore not innovations and have no technological content. Patents are awarded to private enterprises and people who make technological discoveries, as well as to encourage the timely and suitable public disclosure of such breakthroughs. Patent protection seeks to promote technological innovation because it will benefit both the innovators and society at large.Trademarks protect the owner's business from unfair competition and prevent the general public from being deceived about competing products that are available on the market.[3]

**How Is Trademark protection Achieved?**

A trademark can be protected in three main ways by registering it: globally, through the European Union's CTM Registry, or through one or more national registrations. Because they are so widely used in commerce, trademarks are legally protected in many nations. Since these trademarks are not registered with any registry, a search that is both totally accurate and reliable can never be conducted. Trade mark searches are important, nevertheless, since only the owner of a protected mark may use it for goods and services that are identical to or similar to those for which it has been registered. It is also forbidden to use a sign without permission if it has the potential to mislead the public. When selecting a new trademark for its products and services, a company must take into account both legal and elements of marketing. Prior to investing money in promoting the product or service to which the fresh mark will be applied, businesses should be somewhat aware of the possibilities of trademark disputes or other previous rights. This is why researching previous rights has always been a vital part of getting ready to launch a new product or service.[4]

**Infringement of Trademark**

Trademark infringement occurs when someone else utilizes an identical or confusingly similar trademark. Usually, dilution or misunderstanding is involved in this kind of infraction. The term "likelihood-of-confusion" describes circumstances in which a trademark infringement or misleading similarity between two marks could lead to consumer confusion. When a trademark application that is accepted is confusingly similar to one that is already registered, the trademark office frequently objects. The "HORLICKS" trademark was filed in India, according to the Delhi High Court 2002 (25) PTC 126 Del case Horlicks Limited &Ors. Vs. Kartick Sadhukan.This trademark applies to baby and kid-friendly foods and biscuits. A different business, Kartick Confectionery, began producing comparable goods under the "HORLIKS" brand, violating "HORLICKS'" trademark rights. Because of the nature of the products

and how similar the two marks are to one another, the consumer can feel confused. The Delhi High Court's Single Judge Bench, led by Judge B Chaturvedi, determined that HORLICKS is a well-known, respectable brand that holds a dominant position in the industry. Kartick Confectionery was prohibited from using the mark by the Court.If a well-known mark is violated, dilution of trademark is an especially intriguing type of trademark infringement. A well-known brand's business may not be impacted by this trademark usage, but the trademark may be harmed. "Tarnishing" is the use of a trademark in association with improper or sexual content, whereas "dilution of trademark" refers to the process of blurring or tarnishing a well-known brand in order to reduce its distinctiveness and reputation. Blurring usually signifies harm to a recognizable mark. In these cases, pursuing the proper legal action and avoiding dilution can protect the interest in the well-known brand.

In Daimler Benz Aktiegesellschaft vs. Hybo Hindustan, the accused utilized the name "BENZ" on a product that was intended to be used as underwear. Combining BENZ with other products or services is improper due to its broad appeal because it will probably devalue the brand. In her decision, the court stated the following: If the three pointed star that represents the "Mercedes Benz" order is debased by indiscriminate, colourful replication by everyone, including those who produce underwear equivalent to the defendant, I believe there will be a substantial distortion of the law related to Trade Marks and Designs. Nobody is allowed to take that mark or affix it to any goods or objects.(2)

**Trademark Legal Framework in India**

The Indian intellectual property law reflects changes in other countries' IP laws as well as technological advancements. India's trade mark laws were passed in 1999 and are currently in force. The controller of patents, designs, and trademarks, who serves as the last arbiter in trademark registration proceedings, is employed by the Department of Industrial Policy and Promotions of the Ministry of Commerce and Industry of India in India. Mumbai is home to the Department of Trademarks' main office. Nonetheless, the office still maintains its outposts in New Delhi, Ahmedabad, Chennai and Kolkata. The specific law governing trademark registration is the 1999 Trade Marks Act, which took the place of the 1958 Trade and Merchandise Act in September 2003 as a result of TRIPS requirements.(5)The Act aims to tighten trademark protection, expedite the registration process, and prohibit the use of fake marks in commerce, as indicated in its long title. The Act sought to resolve operational problems with the 1940 Trademarks Act and update it to reflect the needs of the country's economic growth. It also consolidated all trademark laws into a single section. As a result, the 1958 Act replaced the provisions of the Indian Penal Code and the Indian Merchandise Marks Act of 1889 regarding the improper use of trademarks.(6)

The Trade Marks Act had a 2010 amendment and addresses both the substantive and procedural aspects of trademark protection, is the body of legislation that governs the Trade Marks Rules 2002.The first to file technique, which does not need prior use of the mark in commerce, is the cornerstone of trademark law. The concept of a trademark has broadened to encompass the registration of designs, including product forms, colour palettes, and packaging. In addition, the protection now lasts for ten years instead of only seven, and it may be renewed for further periods of the same duration. The two most noticeable modifications are these two. The certification, services, and registration were added components that the group marks. Trademarks could only be registered for commodities under the prior laws.The new legislation recognizes well-known trademarks and permits their holders to assert their rights in the case that there is evidence of possible unfair competition resulting from a link between two marks, in addition to products and services that are not covered by the register. Additionally, the present Indian law improved the mark's defence against confusion. Previously, the only way an infringement could be deemed illegal was if it was used to provide the same products or services as the owner. Currently, the owner is able to protect their rights not just in cases where the marks are confusing and could mislead customers, but also in cases where they could be used fraudulently. Any symbol that can be used to visually distinguish one commodity or service from another is considered a trademark in India. Trademarks, product forms, packaging, and colour combinations may all be separately or jointly registered and approved in accordance with TRIPS.The absolute and relative qualifying requirements for signs varied greatly under the Trademarks Act. It is strictly forbidden to use trademarks that are not unique in any manner or that do not differentiate products from services. Those that function

as markers for the kinds, standards, amounts, values, provenance and labels that are commonly used in contemporary discourse. In addition, Indian law forbids the registration of deceptive markings or signs that could mislead the public, violate the faith of an Indian, be scandalous or vulgar, be illegal symbols, or consist solely of signs necessary to produce a technical effect. The characteristics of the goods may also be the source of these significant indications. Signs that are identical to or comparable to a prior to mark pointing to the same or comparable goods or services and that are likely to generate misunderstanding or an unwarranted affiliation are prohibited from being registered under the relevant prohibitions. The Trademark Office has to be notified of International Non-proprietary Names (INNs), as stated by the WHO. Additionally, it is against the law to register names that are identical to or similar to INNs, isolated chemical elements or compounds, other people's names without their permission, and deceased people's names without the permission of their legal representatives. In conclusion, laws like copyright, passing off, or other regulations that forbid using unregistered trade marks in India cannot allow the registration of a trademark if they simultaneously forbid using it for commercial purposes.(5)

## CONCLUSION

In conclusion, trademarks are crucial in modern commerce as they serve as indicators of the product's origin and quality. To safeguard intellectual property rights and prevent unfair competition, it is crucial to register trademarks to protect them.Trademarks can be used to differentiate products or services and aid consumers in making informed purchasing decisions by ensuring consistency and quality. Registered trademarks are protected under India's legal framework and can be defended against infringement and unfair competition. Legal mechanisms are employed to prevent trademark infringement, whether caused by identical or confusingly similar marks. Overall, trademarks are valuable resources for businesses, boosting brand recognition and consumer trust. In order to protect their intellectual property rights and maintain a competitive edge in the market, companies must understand and navigate the trademark registration process.

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**RESEARCH ARTICLE**

# Eco-Innovation in the Market Place: Navigating Green Marketing for Sustainable Success

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**ABSTRACT**

Green marketing is stated to refer to the development and marketing of products or services based on their environmental benefits. With rising awareness among the consumers toward sustainability and environmental issues, businesses have increasingly adopted green marketing strategies to stand out from the rest in a competitive marketplace. This paper discusses the advantages and disadvantages related to green marketing. The advantages encompass a better brand image, more loyalty of the customers, and access to the surging percentages of environment-conscious consumers. Besides, green marketing can offer opportunities for innovation, corporate social responsibility, and ensuring sustainability of the environment. The downside is that undertaking an eco-friendly venture is very costly; risks of green washing are high; and it becomes difficult to measure real environmental impacts of the product. To this effect, the paper looks into the regulations and market conditions under which green marketing proves successful. Gaining competitive advantage and more sustainable goals, therefore, can be addressed by firms using green marketing in their strategies for dealing with some of these challenges.

**Keywords:** Eco-Innovation, Environmental Benefits, Green Marketing, Renewable Resources, Sustainable Materials.

## INTRODUCTION

Green marketing" is the practice of marketing goods and services based on the favorable environmental effects. It means producing and marketing environmentally friendly products, whereby the most negative impact will be

lessened in the production process. Clearly, the above explanation delineates green marketing with examples from today's world for how it can be utilized. Critical Attributes of Environment-Friendly Product and Green Marketing: Green marketing deals with the products that have less unfavourable impact on the environment or are eco-friendly or sourced from renewable resources. Such products may be recyclable, biodegradable, or made from materials processed from renewable resources. A very famous outdoor apparel firm, Patagonia, has underlined sustainability as a strong focus in its business operations. They also encourage customers to repair their gear instead of replacing them and use recycled materials within their products. Their "Worn Wear" initiative encourages customers to repair and resell their used Patagonia products, reducing waste.

Eco-friendly packaging: Of late, growing concerns have been seen on how to reduce the impacts of business ventures on the environment. The latest approach has been using eco-friendly packaging solutions such as avoiding all plastic usage, which is avoidable through biodegradable material usage, or shrinking down the sizes. An example is Unilever, which states that all its plastic packaging will either be compostable, recyclable, or reusable by 2025. The company has also launched brands such as Love Beauty and Planet, whose ingredients are sourced sustainably. The bottles used are made entirely from recycled plastic.

**Green Labels and Certifications:** Many companies seek to acquire the labels and certificates that align with their environmental claims. These certifications can help increase consumer confidence and distinguish products in the market. A well-known example is the Energy Star certification program, which labels electronics and appliances as energy-efficient. The U.S. Environmental Protection Agency has strict requirements for these labels regarding energy efficiency. Customers concerned about the environment will be excited by a full range of Energy Star-certified offerings from companies such as LG and Samsung. Transparency and Education: Green marketing companies often tend to educate the consumer on the environmental impacts of their products. This may include transparent sourcing and manufacturing practices, informative labelling, and clarity of labels. For instance, the Seventh Generation eco-friendly household brand emphasizes transparency in marketing. The firm offers to all customer's extensive information regarding the composition of the product as well as the environmental effects of the product in support of effective decision-making. Corporate Social Responsibility (CSR): Many firms have embedded green marketing into their overall CSR to prove they indeed care for sustainability in ways that relate to products. A case in point is IKEA's recent announcement: to become climate positive by 2030. They are encouraging consumers to recycle and reuse products, supporting sustainable material sourcing, and making investments in renewable energy. Among their initiatives are furniture made from sustainable materials and furniture recycling services.

**Significance of the Study:**

The relevance of studying green marketing lies in its depiction of the emerging trend of environmentally conscious consumerism. Green marketing promotes products that are 'eco-friendly' as it caters to the increasing demand for sustainable options. One of the strengths is the enhancement of the brand image of companies and attracting environmentally conscious customers and, thereby gaining a better strategic position over competitors. In addition, green marketing promotes innovation by forcing companies to innovate their products to be more sustainable and reduce their environmental footprint. However, it also presents risks, such as higher costs of production for green products, which, in turn, will affect the price and profitability of those products. The risk of brand credibility degradation through greenwashing, whereby companies exaggerate their eco-friendly nature, lies in wait. The consumers have to be educated that such a thing as green exists because sustainability value can be nebulous when the consumers do not know what being eco-friendly means. It is also complicated to balance the profitability and the sustainability of green marketing. However, long-run advantages include customer loyalty and environmental stewardship. For this reason, green marketing is an important field of study in sustainable business growth.

## REVIEW OF LITERATURE

1. Alkhatib et al. (2023) describes green marketing in the digital age, which bears benefits such as awareness and knowledge on the part of the consumer concerning sustainability through online platforms. For instance, with the challenges posed by this rapid change in technology and finding a way to wed digital strategies with sustainability goals, there are several advantages, including increased consumer awareness and knowledge concerning sustainability through online platforms.
2. Kiyak and Grigoliene (2023) talked about the fundamental concept of green marketing by referencing the 4P marketing mix. According to the study, green marketing, which essentially is presumed to enhance brand perception and boost demand for 'eco-friendly' products, is an indispensable measure to propel business. However, the burden of such venture in terms of green material and technology is a supplementary cost.
3. Kiyak, D., &Grigoliene, R. (2023). Conceptual frameworks of green marketing: an analysis. Sustainability, The paper uses the 4P model to discuss green marketing and identifies advantages in regard to environmental stewardship but underscores financial risks from investment in sustainable innovation.
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**Statement of the problem**

This increasing adoption of green marketing and also gap in its actual effectiveness and sustainability, as it brings gains on one hand but has its own level of challenges on the other. Though green marketing can take an advantage of the scope through brand reputation and consumer loyalty and increased corporate responsibility, its barriers include the cost of producing and the risk of greenwashing, due to which consumers are afraid to trust it. Since the product makes claims on sustainability, accurately measuring the environmental impact of that product is quite challenging and irregular. This paper thus looks into how green marketing strategies can be implemented within regulatory frameworks and market dynamics to identify the routes via which companies could break the barriers and implement the most smooth and efficient positive impacts of green marketing on both firm performance and environmental sustainability.

**Objective of the study**

This conceptual research analyses the benefits and challenges of green marketing, as important to highlight its role in enhancing brand image, building consumer loyalty, and attracting environmentally-conscious consumers and

implementing green marketing comes with challenges, challenges must be addressed to fully leverage the benefits of green marketing.

**Analysis**
**Benefits of green marketing**

**Improved perception of the brand**
The reputation and brand image of a business are greatly enhanced by green marketing. Customers are demonstrating a strong preference for brands that exhibit environmental responsibility as environmental concerns gain prominence. The way businesses approach their marketing strategies and general business practices is changing as a result of this shift in consumer preference The outdoor apparel brand Patagonia is a perfect illustration of this. Patagonia has integrated environmental sustainability into its fundamental business model, going beyond just offering products. This has helped to establish the company's brand. The "Don't Buy This Jacket" campaign, introduced by Patagonia in 2011, ironically increased sales and brand loyalty. This campaign asked people to think about how their purchases would affect the environment, even if it meant avoiding Patagonia goods. Patagonia's brand image was greatly improved when they showed a genuine commitment to sustainability by putting environmental concerns ahead of immediate sales.

Unilever's Sustainable Living Plan is an additional illustration. The corporate strategy was initiated by the company in 2010 towards helping to almost double its sales while reducing the corporation's environmental footprint by 50%. In 2019, the firm revealed that Sustainable Living Brands had accounted for 75% of the company's growth and had performed better than the rest of the business at 69%. In other words, a commitment to sustainability can work to make a difference in the brand image and induce business growth. Positive and anecdotal results on brand image are the benefits of such green marketing strategies. A Journal of Business Ethics article notes that green marketing strategies help enhance the brand perception that, in turn, motivates a customer to seal the deal. This paper provides empirical evidence that propounds a positive relationship between green marketing and brand perception. Furthermore, in a Journal of Marketing Management paper, application of green branding strategies results in better brand attributes. The authors of the study conclude this positive brand attitude results from both functional attributes- such as environmental-friendly product aspects- and emotive benefits- such as feeling good about supporting a green brand.Mendleson and Polonsky (1995)

**Effective Customer Retention**
This alignment would help customers feel that the brand shares the same values, hence intensifying emotional attachment and inducing repeat business. LUSH Cosmetics presents a very effective practical example of this concept in action. LUSH has attracted a very loyal customer base by trying to maintain environmentally-friendly policies. They source responsibly, sell products "naked" or in reusable packaging, and now attempt packaging with minimal materials. The customers supporting these policies have become a very loyal following for the company. Companies that always conform their practices and products to the values of the environmentally conscious consumers are likely to be characterized by very trusting relations and loyalty among customers. Seventh Generation, for example, is a green company that manufactures personal care and household products. Due to its commitment to recyclable packaging and plant-based ingredients, the company has managed to create loyal following. Factful disclosure of what it uses for its ingredients and practices has cemented the loyalty of the customers towards the company.

It is established that green marketing has a considerable relationship with customer loyalty in academic research. In fact, according to a published article in the International Journal of Contemporary Hospitality Management, green marketing techniques had significant influences on customer loyalty, especially from environmentally concerned customers. According to the study, the customers would tend to have a long-term bonding with the brand if they perceive that the brand is really concerned with environmental sustainability. In addition, articles published in Journal of Cleaner Production, established the concept of "green trust" as a mediator of green purchase intentions, which connects green perceived quality, perceived risk, and green satisfaction. This study identifies how important it

is to develop the relationship with trust with customers through real and integrated green marketing methods with consumers. Rizka et al (2020)

**Market Differentiation**

Green marketing offers the ability for businesses to differentiate themselves over the competition. If businesses can provide environmentally friendly products or engage in sustainable activities, they may attract customers who, in turn, care for sustainability. In many competing markets, businesses may set themselves apart if they offer products which are friendly to the environment or engage in sustainable activities. Tesla is a classic example of how to use the concept of green marketing successfully to individualize a product in the market. The company has segregated itself in the automobile industry by simply making electric cars. Considering tough competition from established auto manufacturers, Tesla has gained substantial market share and developed into a leader within the EV space because of its business approach being eco-friendly. Method: An example is a consumer goods company that specializes in making cleaning products. In the market for cleaning products, Method distinguished itself with its provision of environmentally friendly, biodegradable cleaning solutions in chic, recyclable packaging. Using this strategy, Method has managed to carve out a niche for itself in a market dominated by the conventional cleaning product brands. A recent study in the Business Strategy and the Environment journal indicated that business organization competitiveness is improved through creating green products. The study shows that an investment in green innovation enabled businesses to differentiate their products, which in turn improved market share and profitability. Beyond this, some in the Journal of Business Ethics have researched how and why businesses integrate environmental sustainability in their new product development. Researchers further found thatbusinesses could differentiate effectively in the market and create a competitive advantage through sustainability integration during new product development.

**Cost saving**

Focusing on green practices usually translates directly into cost savings through increased productivity and resource conservation. Although the initial investment may be higher, long-term savings may be enormous once green technologies or processes are adopted. A perfect example of how green initiatives save one dollar is with Walmart's sustainability program. The company has almost saved a billion dollars annually in terms of potential savings through its packaging reduction and efficiency of fleet. It saves dollars through such cost savings as less fuel used, better material utilization, and more efficient logistics. Another strong example is the international commercial flooring company Interface. Through the Mission Zero initiative, which meant the reduction of any damaging impacts on the environment by 2020, the firm reduced its water intake by 83% per production unit and the energy use by 39% per unit. It would thus achieve a huge cost savings due to the efficiency improvement it was making.

According to a study published in the Journal of Cleaner Production, environmentally responsible methods of manufacturing can become considerably cost-effective and profitable. "These practices-conservation of energy, reduction in waste generation, and optimum use of materials-have led to lower operating costs besides mitigating adverse effects of these on the environment," the researchers have argued. Published works in the Journal of Environmental Management indicate that proactive environmental policies can enable organizations to attain greater cost effectiveness and gain superior environmental performance. For example, business ventures that voluntarily implement environmental management systems often experience improvements in operational effectiveness and lower compliance costs.

**Access to New Markets**

In turn, new markets are opened to businesses that employ green marketing techniques because of the increasing awareness among consumers of sustainable products. Now, new market segments with a willingness to pay premiums for environmentally friendly goods and services are being identified as consumer awareness of environmental concerns increases. A firm such as Beyond Meat manufactures plant-based meat alternatives, which have gained entry in the growing realm of health-conscious and conscious environmental customers. The fact that their products are being sold over 80 countries indicates the popularity of sustainable food in the world. Since

Beyond Meat has entered products that target and mitigate health and environmental issues, the firm has been able to penetrate a given market at a rapid rate. Ecover is a Belgian company that deals in cleaning products that are genuinely eco-friendly. It has taken advantage of the increasing demand for environmentally friendly household items, which made it grow from a niche market in Europe to a global brand. It is also mentioned that new market niches can be created and market share increases can happen through innovative green products, as a research study shows that the findings of the study were that businesses which were capable of successfully designing and marketing environmentally friendly products have shown a significant chance of welcoming new customers and uncovering hidden markets. Furthermore, a paper from the Journal of Cleaner Production explored how eco-innovation fosters market niche development. The findings from the study indicate that companies that invest in eco-innovation have a better chance of detecting and benefiting from new sustainable market trends. (Cronin et al., 2010)

**Compliance with Regulations**

With green marketing, companies will be positioned more gracefully ahead of environmental regulations and avoid fines and penalties for non-compliance. In short, proactive green marketing strategies will be in place to position the companies well against the growing list of stringent environmental regulations in the world. Apple's commitment to power 100 percent of the company's business operations on renewable energy enhances the environmental positives and ensures that their operations remain within the bounds of always tightening environmental regulations around the world. In 2018, Apple went beyond the demands of many jurisdictions' regulatory requirements as the company fulfilled its commitment to using 100 percent renewable energy in powering all of its facilities. On the other hand, Volkswagen offers a warning. The company's emissions scandal in which it was discovered to have cheated on emissions tests caused massive reputational harm and fines totalling billions of dollars. The significance of true green practices and the dangers of non-compliance are brought to light by this case.

Proactive environmental strategies can assist businesses in improving their environmental performance and regulatory compliance, according to research published in the Journal of Environmental Management. According to the study, businesses that voluntarily implement environmental management systems are more likely to meet compliance requirements and are better equipped to handle new regulations. Additionally, a study published in the Strategic Management Journal looked at the connection between firm performance and environmental performance. The researchers discovered that better environmental performance can result in better financial performance, in part because of lower risks and expenses associated with regulations. Jain and Kaur (2004)

**Positive Impact on the Environment**

Green marketing has advantages for business and environmental sustainability. Businesses that put an emphasis on environmentally friendly practices contribute to resource conservation, pollution reduction, and a healthier planet. The global commercial flooring company Interface, which was previously mentioned, has made a commitment to become carbon neutral by the year 2040. Since 1996, their "Climate Take Back" campaign has already led to a 96% decrease in the intensity of greenhouse gas emissions. This demonstrates how a corporate's commitment to sustainability can have a significant positive impact on the environment. Examples of green marketing that actually aid the environment are Patagonia's Worn Wear program. The company supports customers to repair and reuse their Patagonia equipment, thereby reducing waste and purchases of new equipment. This becomes highly helpful in an environmental sense and also adds to the reputation for the brand.

The firms that adopt green marketing practices may critically reduce their environmental impacts associated with the lifetime of a product, according to a study published in the Journal of Cleaner Production. It concludes that the firms that often use the designs of products that entailed recycling or disposal are easier to recycle or dispose of, along with their more environmentally friendly materials and more sustainable production processes. A study in the Journal of Business Ethics investigated the environmental impacts of green innovation. According to the study, the company not only had improved its environmental performance but had to regularly prod its suppliers and customers to become more environment-friendly, thus producing a knock-on positive effect on the environment. Purwanti et al. (2019)

**Attracting Investment**

This is also attributed to the fact that investors are increasingly investing in companies with sustainable practice policies. In any case, the growth in ESG (Environmental, Social, and Governance) investing has made environmental practices an important consideration when making investment decisions. A Sustainable Living Plan, in which Unilever aims to decouple its growth from its environmental impact, increases investor interest. Comparing shareholder return, Unilever has always beaten its peers, showing monetary profit from a good sustainability commitment.

For instance, Tesla has also managed to raise a lot of investment despite having losses in business for years mainly because of its aspect of environmentally friendly transport. This is because the Company, which can easily change the face of the car-making sector, has seen investors fighting to fund this venture. Research published in the Journal of Business Ethics suggests that firms with high quality ESG practices have far lesser reduced capital constraints and attract much higher investments. This study suggests that companies with better environmental sustainability performance are under much lesser capital constraint; thus, investors are likely to invest in more environmentally friendly businesses. Lastly, an article in the Journal of Banking & Finance presented research that studies the relationship between the cost of capital and corporate social responsibility or CSR. The authors found that firms with better CSR performance have lower equity capital costs, thereby indicating that investors perceive such firms as less risky. Berrou et al. (2019)

**Challenges in green marketing:**

**Lesser awareness of consumers**

The rational choice theory says that a consumer, being an economic agent, is expected to maximize utility through value and cost calculus in whatever choice he or she makes (Plottu&Plottu, 2007). In evaluation, however, it leaves most of the time to ignore consideration of environmental cost and benefits, hence such consumer behavior turns not aligned with sustainability from an environmental sense. As can be perceived from practices in certain tribes and religious groups, the cultural norms and belief systems affect consumption patterns to a great extent. In this context, people usually opt for short-term benefits at the cost of long-term environmental costs. Furthermore, green products are also perceived as inferior, which further creates an attitude among consumers and enhances the decision-making process in purchasing goods, thereby delaying the acceptance of the sustainable consumption practice (Pham et al., 2019). In other words, individuals lacking a total overview of information make consumer decisions much more complicated; they could not possibly account for the long-term outcomes of their consumption. This fact betrays the attachment towards incorporating environmental considerations into consumers' decision-making in making resistance to cultural belief, social norms based on the aforesaid challenge that hinders the development of sustainable consumption practice.

The gap between the green behavior by the consumers and the perception of its long-term consequences for the environment. Such a common factor as environmental resources makes a person feel less incentivized to protect them, and hence, this consciousness endorses the idea of Earth's inexhaustible resources. It makes it hard for people to believe in the seriousness of possible damage it may cause. Further, skepticism toward environmental issues like global warming and climate change could minimize risks associated with environmental crises for consumers, and they can trivialize such risks (Whitmarsh, 2011). In case consumers do not realize individual actions contributing to collective outcomes for the environment, then efforts in green marketing might be thwarted, and changes toward eco-friendly products might be also impeded. Taddress the mentioned challenges, the knowledge gap needs to be bridged and consumers need education on the environmental implications of their choices. This would ensure that the consumers made the choices in a value-toward way and leaned towards environmental sustainability. Eventually, this would shift the cultural barriers within sustainable consumption practices, thereby reducing the environmental implication of consumers' behaviour

**Government**

Governments are at the center of pushing forward this agenda towards sustainable development. They are in charge of policy-making, regulatory policies, and transformational leadership for purposes of sustainability (Van der Waldt, 2016). In 2015, the UN Sustainable Development Summit was a great culmination with 193 countries committing themselves to the ambitious 17 SDGs and 169 targets. National governments played a monumental role in formulating this global framework. A few others have encountered significant success through carbon taxes and congestion levies, while others face leadership, a regulatory framework, and commitment barriers. Indeed, in many countries, what has mainly hindered progress is short-term political considerations, misaligned policy priorities, and a lack of intergovernmental collaboration (Bowen et al., 2017).

The interconnectedness of the biophysical environment puts forward the fact that local actions have global implications. Pollution in one country affects faraway lands. Although cooperative frameworks exist in areas such as finance and economics, to date, most national governments lack a similar collaborative effort toward sustainable development (Polonsky, 2011). Political parties often prioritize short-term gains to maintain power, leading to the politicization of policies, a trend particularly detrimental to sustainable development efforts in developing economies. The absence of a standardized framework for assessing governments' sustainability performance, unlike the financial risk ratings used to evaluate government borrowing. This deficiency can be attributed to the precedence given to short-term economic and financial outcomes over long-term environmental risks, which can have severe negative consequences.

**Challenges Faced by Firms in Economic Exchanges**

In any economic exchange, the primary role of a firm is to deliver value to the marketplace while simultaneously generating value for its shareholders. Additionally, firms play a critical role in society by providing continuous employment and ensuring favorable working conditions. However, firms are often confronted with conflicting expectations on one hand, they must focus on creating economic value, while on the other, they are increasingly pressured to integrate societal and environmental goals into their overarching strategies. Traditionally, performance assessment has been predominantly centered around profitability. As a result, firms tend to prioritize projects with positive net present values that offer immediate financial returns to satisfy shareholders. Although there have been efforts to adopt multi-dimensional performance frameworks, such as the Triple Bottom Line (TBL) model which emphasizes profit, people, and the planet, companies often revert to financial measures as their primary benchmark (Norman & MacDonald, 2004). Moreover, financial incentives like bonuses for top management are typically tied to profitability. Notably, the biophysical environment has yet to be formally acknowledged as a stakeholder with representation on corporate boards.

Society as a whole has not yet fully embraced the importance of social and environmental performance, nor has it reconsidered how firms should be evaluated. Both individuals and organizations contribute to this economic system, and a shift in attitudes is imperative. Furthermore, firms encounter operational challenges in adjusting their marketing mix and adopting appropriate segmentation, targeting, positioning, and differentiation strategies (Kotler & Armstrong, 2014). The core marketing elements product, price, place, and promotion require modification to align with green marketing principles (Dangelico &Vocalelli, 2017).

Incorporating sustainability into a firm's value chain necessitates a life cycle assessment, encompassing every stage from product design and development to production, promotion, consumption, and disposal (Hasan et al., 2019). Firms that do not appreciate the strategic value of sustainability often resort to "greenwashing," that is, firms that falsely portray their business practices as environmentally sound (Delmas & Burbano, 2011). On the other hand, firms that create a corporate culture with a sincere commitment to environmental sustainability have a higher chance of obtaining excellent operational and green marketing performance, hence gaining a competitive advantage in green market segments (Moravcikova et al., 2017).

**Availability of green finance**

Green financing and investment opportunities form a significant aspect of furthering the pursuit of SDGs. In this context, the World Economic Forum (2013) suggests that an additional $0.7 trillion every year was needed to entirely satisfy worldwide clean energy as well as climate-smart infrastructure demand, more than doubling since that estimate. However, the high uncertainty in green business sectors and the lack of historical return data make investors hesitant to fund these ventures (Al Breiki &Nobanee, 2019). Despite this, sustainable practices have emerged as profitable business strategies. Firms adopting environmental management, such as using recycled materials, often see a rise in stock prices and generate positive abnormal returns (Yadav, Han & Rho, 2016). To address funding challenges, partnerships between governments and private investors are necessary, especially in developing countries where financial resources are constrained. Though initial investments in green ventures may yield lower returns, consumer attitudes are shifting, with increasing willingness to pay for sustainable products. In the long term, these investments are expected to be rewarding. (Risalvato, Venezia & Maggio, 2019). Ultimately, fostering research and innovation in green products is crucial, and access to funds for green business development will support broader economic and environmental goals.

## CONCLUSION

Numerous advantages come with green marketing, including increased consumer loyalty and brand image as well as the ability to enter new markets and draw in investment. Businesses that implement green marketing strategies are likely to see growing returns on their sustainability investments as environmental concerns continue to grow globally. But it's imperative that businesses make sure their green marketing initiatives are sincere and significant. Disingenuous environmental claims, or "green washing," can seriously harm a company's brand and outweigh any possible advantages of green marketing. Going forward, as we strive for a more sustainable future, green marketing will probably become an essential requirement for companies in every industry, rather than just a differentiator. Businesses who fully and early adopt this change stand to gain a great deal in the long run as well as make a positive impact on a more sustainable world. Green marketing is about more than just attracting eco-aware customers, as the examples and research in this analysis show. It's a thorough approach to business that can boost productivity, stimulate new ideas, and result in sustained success. We may anticipate a continuous movement across industries toward more sustainable business practices as more companies become aware of these advantages. Kumar (2015).

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**RESEARCH ARTICLE**

# Impact of Spiritual Intelligence on Burnout and Job Satisfaction of IT Employees

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**ABSTRACT**

The aim of the study is to analyse the impact of spiritual intelligence on burnout ad job satisfaction of IT Employees. The present study is descriptive research design. Based on the Kregcie-Morgan table 109 eligible responses for the study were considered based on convenience sampling by drawing on prior research and adding pertinent demographic questions, a well-structured questionnaire was created The information was gathered by means of a survey and Google Forms. Factor analysis in SPSS and confirmatory factor analysis in AMOS were used to interpret the data. The results of the study show that Spiritual intelligence increased job satisfaction and reduced Burnout. Researchers also discovered that individual having high level of spiritual intelligence feels less stress and seems finding work more sense to enjoy. Spiritual practices like mindfulness, mediation, and maintaining a gratitude philosophy can be woven into the day to give IT employees the ability to go inward and outward more freely while traversing the waves of technology changes without riding them. Further, creating a culture that encourages support and feels inclusive of the importance of wellness for faculty can also serve to decrease burnout and enhance satisfaction in their work.

**Keywords –**Emotional Intelligence, Information Technology, Mobile Telecommunications, Psychological Ownership, Spiritual Intelligence.

## INTRODUCTION

The agrarian, industrial, information, and technological revolutions came first, second, and third, respectively, throughout human history; the fourth and last wave of revolutionary civilisation will be based on spiritual predisposition(King, 2009). People lose motivation and start seeking meaning in things other than monetary possessions when the economy is in a slump. They begin to seek the significance and goal of their existence

(Srivastava, 2016). Workplace spirituality is gaining prominence in today's cutthroat business environment. If it wants to stay ahead of the competition, every company is searching for ways to set itself apart. Emotional and logical intelligence alone won't cut it. A more focused pursuit of purpose and meaning in life can help us navigate the challenges of the modern world. Rather than focusing solely on a candidate's technical abilities, business executives are beginning to see the full value of each individual (Nazir & Nazir, 2018). More and more business schools are incorporating spirituality into their curricula, employees are seeking deeper meaning in their lives and the lives of those around them, and as a result, businesses are fostering more compassionate work environments' (Safara & Bhatia, 2013). In a number of studies, Spiritual intelligence and Job satisfaction have been found to be correlation. Employees with high spiritual intelligence ratings tend to have a high level of job satisfaction, employees seem more likely to enjoy their jobs if their spiritual intelligence is also high (Purwanggono, 2023). This is a function of the feeling of purpose and fulfilment that spiritual practices bring to one's life. It allows individuals more positive thinking in their jobs and overall satisfaction with their work and even greater due to this. With the continuing growth and transformation of India's IT industry, cultivation of spiritual intelligence in its employees may become a key factor in keeping up high levels of job satisfaction together with increasing productivity.

When employees increase their Spiritual intelligence, the level of burnout in the Indian IT industry goes down. This is because employees possessing higher spiritual intelligence can manage stress more effectively; a positive mind-set comes more naturally to them, they are able to find more meaning and purpose in their work. In the face of challenges, they possess the ability to keep getting back up and are able to maintain a work-life balance (Urs,et al., 2024). This in turn means that they experience higher job satisfaction, with rates of turnover and absences all dropping significantly for most kinds of tension in workplace management—the latter especially so in industries where stressful work is common.Indian IT businesses get a real dividend from nurturing spiritual intelligence among employees (Usmani et al., 2024). By making the wellbeing and fulfilment of employees a priority, companies not only create an atmosphere that promotes good working conditions but also enables people to thrive and flourish. As a result, both levels of staff burnout go down; manger retention rates improve and more employees perform better their jobs in general way than before. Spiritual Intelligence is certainly playing a key role in deciding the future shape of India's IT industry (Farah et al., 2023). With this background the current study aims to analyse the impact of Spiritual intelligence on Jobs satisfaction and Burnout. The first section of this paper deals with brief introduction, the second section elaborates the review of literature and identifies the research gap. The research methodology specifies the methodology used for the study and the fourth section discusses the results and discussion. The final section presents the conclusion and scope for further research.

## REVIEW OF LITERATURE

Academic papers from the past that were pertinent to the research concerns under investigation were examined in this study using a systematic literature review methodology. We collected the articles from respected academic journals and carefully examined them to determine the quality of each study. Elsevier, Routledge, CRC Press Taylor & Francis, and others are the databases that are cited. This research made use of data from three databases: Sage, Emerald Group Publishing, and Springer Nature. The following reputable academic databases were consulted for the acquisition of supplementary scholarly articles: Wiley, Academia, JSTOR, and Guildford Press.

According to Urs, et al., (2024) Workers who have developed spiritual intelligence (SI) are more equipped to find purpose in the face of adversity. Because of this, people are seeing the COVID-19 pandemic in a new light and are making the most of their situation. SI encourages workers to think outside the box, act compassionately, change their views, and use their observations to spark creativity.Usman et al., (2024) found that Spiritual intelligence and work-family conflict were important predictors of job performance among married female university professors. The results also showed a correlation between spiritual intelligence and work-family conflict and job performance. This study provides comprehensive data on teachers' job performance, which will serve as a driving factor for educators to enhance their performance on the job.According to the results of Farah et al., (2023)spiritual intelligence does

influence work satisfaction, although stress on the job has little bearing on the links between spiritual understanding and job contentment. This research contributes to the growing body of knowledge in the area of hospitality management, particularly as it pertains to spiritual intelligence and the correlation between stress and contentment on the job.In the study of Purwanggono, C. J. (2023). Emotional intelligence does not impact performance negatively or significantly, and intellectual intelligence does not either on employee performance. In addition, a worker's productivity at Telogorejo Hospital is positively impacted by spiritual intelligence and statistically significant. The findings of Masitoh, S., & Sudarma, K. (2019) demonstrated that spiritual intelligence and emotional intelligence, even when measured in part, significantly impacted both employee performance and job happiness. Job happiness is positively and significantly impacted by employee performance. Job happiness is mediated by employees' emotional and spiritual intelligence through their performance on the job. There is a lot of evidence that shows how important it is for businesses to provide training in emotional and spiritual intelligence in order to boost employee performance and happiness on the job.Heydari et al (2017) expressed that managers should think about implementing spiritual intelligence training for nurses as a means to boost employee happiness on the job.Korankye and Amakyewaa, (2021) expressed thatEmployees' levels of job happiness and their intents to leave are both positively affected by emotional intelligence, according to the study. Study participants in the mobile telecommunications industry reported higher levels of job satisfaction and lower levels of intention to leave their current positions as a result of their spiritual intelligence .Tampubolon, et al., (2021) emphasised that Employees' ability to connect with clients on an emotional and spiritual level is crucial in the banking industry. The employee's social capability also plays a role in this, so that he or she aims to be satisfied with their employment in the end. A study by Sunaryo et al., (2017)showed that spiritual intelligence lowers the risk of burnout on the job, emotional and spiritual intelligence raises the likelihood of caring behaviour, and burnout lowers the likelihood of caring behaviour. Spiritual intelligence and compassionate behaviour were found to be the best predictors of job burnout. When it comes to forecasting job burnout and caring behaviour, spiritual intelligence would be more relevant than emotional intelligence. This discovery has the potential to inspire future studies because of the benefits it could bring to patients and nurses alike.

Kaur et al., (2013) revealed Several factors influence the caring behavior of nurses: burnout affects their emotional intelligence, which in turn affects their psychological ownership. Psychological ownership, in turn, mediates the relationship between spiritual intelligence and caring behavior. Finally, burnout is a mediator between emotional intelligence and psychological ownership. Lastly, psychological ownership mediates the relationship between burnout and caring behavior. Moradi et al., (2017) expressed that between spiritual intelligence and job burnout, there was a notable negative correlation. In addition, there was a very unfavourable correlation between psychological capital and job burnout in the personnel. Spiritual intelligence may have an effect on employee burnout via psychological capital, according to the findings. Reducing burnout on the job is possible through spiritual intelligence and psychological capital. Thus, it would be beneficial for employers to enhance various psychological characteristics, such spiritual intelligence and psychological capital in particular, in order to create a more vibrant work environment and decrease job burnout.Zhaleh, K., & Ghonsooly, B. (2017) revealed that there was a statistically significant inverse correlation between the spiritual intelligence and burnout levels of the EFL teachers. Also provided were the correlations between the various variable subscales. When looking at spiritual intelligence and burnout, the results showed that the genders of the participants were not significantly different.Ardhani and Aini, (2022) expressed that Hospital nurse burnout, which is impacted by spiritual intelligence and spiritual leadership, as well as other factors that were not considered in this study accounting for 79% of the total. There is a clear correlation between spiritual leadership and a decrease in nurse burnout. Nurses' low rates of burnout are unaffected by spiritual intelligence. When spiritual intelligence is coupled with spiritual leadership, it significantly reduces nurse burnout.

Spiritual leadership, vocation, emotional intelligence, and teacher burnout were discovered to have a strong inverse relationship, according to Li et al. (2023). A person's spiritual leadership had a favorable and statistically significant effect on their EQ and their sense of vocational calling. Spiritual leadership and teacher burnout were also mediated by emotional intelligence and professional calling. Our understanding of the connection between spiritual leadership and teacher burnout is enhanced by the findings, which also provide light on the mediation roles of vocational

calling and emotional intelligence in this equation. Primary and secondary school administrators can benefit from spiritual leadership styles in the fight against teacher burnout if they inspire their staff to pursue their interests and grow emotionally intelligent, according to the research. Ismail et al., (2020) expressed that Emotional, social, physical, spiritual, and resilience intelligence models with burnout resilience have achieved concurrence value, according to the study's findings. A teacher's ability to inspire greatness in their students depends on his or her own brilliance and resilience in the face of adversity. For stakeholders like the Malaysian Ministry of Education, who are trying to find better ways to choose teachers and provide them with training to avoid burnout, indicators of emotional, spiritual, physical, and burnout intelligence are crucial. According to Mirshahi, R., & Barani, G. (2016) It would appear that spiritual intelligence plays a significant role in both our personal and professional lives, and that this is a key factor in the success of educational organisations and, by extension, in the professional lives and societal roles of teachers. Also, it's not easy being a teacher in this day and age. A major issue in today's education is teacher burnout, which can be caused by stress at work. Teacher performance in the workplace might be negatively affected by job fatigue.Workplace burnout negatively impacts both teachers' happiness and their ability to do their jobs well. Furthermore, this could have repercussions for the well-being, productivity, and health of the classroom instructors.

There are many different studies about the role of spiritual intelligence in job satisfaction and burnout within the medical sciences and education industry. However, while this may be important, it is much less certain within IT Employees who may also be the most vulnerable to stress and burn out from the depersonalizing advice that their workload gives them in order to cope with it. More research is clearly needed to be able to understand what this means and how it can be used to help IT professionals with their mental health and job satisfaction.

**The objective of the study**
To study the impact of SI on Job satisfaction
To study the impact of SI on Burnout

## RESEARCH METHODOLOGY

The present study is descriptive research design (Siedlecki, 2020). One hundred participants were selected for the study with a 7.5% margin of error, according to the Kregcie-Morgan table (Krejcie, 1970). We circulated 150 questionnaires and received 109 eligible responses for the study. Convenience sampling was employed for the sampling process (Golzar et al., 2022). The convenience sample method was selected because it provided a simple and easy technique to collect data from employees in the IT business. By drawing on prior research and adding pertinent demographic questions, a well-structured questionnaire was created refereeing to The Spiritual Intelligence Self-Report Inventory (Sisri 24) (Wan et al., 2017), The Burnout Measure (Short Version), Malach-Pines, A. (2005), and Sinval, J., & Marôco, J. (2020). Short Index of Job Satisfaction. In order to get honest and insightful answers, the questions were framed in a way that would not introduce prejudice. To further guarantee the questionnaire's validity and reliability, it was evaluated by specialists in the field. Additionally, the reliability and validity of the survey were examined by referring to the Gaskins master validity table. All four constructs had an AVE over 0.500, while composite reliability and Cronbach's alpha were both over 0.70. Since the interitem correlations are smaller than the square root of the AVE, the discriminating validity was confirmed (Louangrath & Sutanapong, 2018).The information was gathered by means of a survey and Google Forms. The survey may be distributed easily and data collected efficiently using the Google Forms platform. Accurately collecting and analysing responses from a large sample size was made possible by the procedure (Sürücü & Maslakci, 2020). Factor analysis in SPSS and confirmatory factor analysis in AMOS were used to interpret the data (Gallagher et al., 2008).

## RESULTS AND DISCUSSION

**Profile of the IT Employees**

In order to accurately analyse and understand the collected data, demographic characteristics must be included. Researchers can use this approach to identify possible differences in opinion based on demographic factors like age, gender, race, income, level of education, and location. By understanding the demographics of the study's respondents, researchers can make better educated conclusions and ensure that their findings are more representative.46.8% of IT employees are female and 53.2% are male in the current study. This finding suggests that there is a relatively equal distribution of gender within the IT Industry. However, it is important to note that gender disparities may still exist within specific roles or levels of seniority within the IT department. Further research is needed to explore the reasons behind these gender imbalances and to implement strategies for promoting gender equality in the IT profession. 29% were below the age group of 30 years, 47% were in the age group of 31–40 years, and 24% were above 41 years and up to 60 years. The qualification revealed that 70.6% of IT employees in the study are postgraduates. 66.1% of IT employees are married, and 28.4% are single. 15.6% of IT employees had less than 2 years of experience, 22% had 2–5 years of experience, and 49.5% had more than 8 years of experience.

**Item analysis for Spiritual intelligence, Burnout and Job satisfaction**

The mean scores above 3.00 indicated that the IT Employees have moderate agreement to Critical Existential Thinking (CET) (M = 3.12, SD = 0.876),Personal Meaning Production (PMP) (M = 3.07, SD = 1.100), Transcendental Awareness (TA) (M = 3.37, SD = 0.765). and Conscious State Expansion (CSE) (M = 3.21, SD = 0.765). The standard deviation for all statements of Spiritual intelligence factors is below 1.000, indicating a small variation in the opinions of IT employees. The skewness and kurtosis are well within the established threshold of -3.00 and +3.00, indicating a normal distribution of data. In total, outcomes conclude that IT employees express similar agreement to Critical Existential Thinking, Personal Meaning Production, Transcendental Awareness and Conscious State Expansion. The small standard deviation and the normal distribution of data suggests that there is not a significant difference in values between IT employees in terms of these spiritual intelligence factors. The uniformity of answers might be more indicative of a common mindset or ethos within the IT team.

Burnout items had a mean score of above 4.000 indicating higher burnout among IT Employees and Job satisfaction is below 3.50 indicating moderate job satisfaction among IT Employees. Burnout indicators had a mean score of 4.000 or more, that is, a higher level among IT Employees However, job satisfaction was still rated in the range below 3.50-an indication that students remain about moderately satisfied as a whole. This tantalizing tidbit hints that IT Employees are not only badly overwrought but also of moderate assurance in the job they're bebusy doing. Many employers underestimate this to some degree or another-and, as a result, should not be paid an hour's worth of wages. It is beneficial to employers that they get to the root causes of burnout and so strive for improvements in job satisfaction, in this way ensuring healthy productive workplaces for their IT Employees. Employing such strategies as offering mental health resources, promoting work-life balance, and recognizing employee achievements can help alleviate burnout and increase job satisfaction in the IT industry.

**H1- Impact of spiritual intelligence on Burnout and Job satisfaction**

**Exploratory factor analysis for Spiritual Quotient**

The KMO measure of sampling adequacy is 0.878, and Bartlett's Test of Sphericity shows a significance level of 5%, indicating strong statistical significance. The chi-square analysis revealed a Chi-square value of 4591.87 for the Bartlett test, with a significant value less than 0.05. This indicates that the correlation matrix is not an identity matrix, suggesting it is indeed factorable. Communalities indicate the extraction values for each item, and it's great to see that they should be above 0.300. The communalities for SQ items ranged from 0.512 to 0.898, which is quite promising.The total of squared loadings that has been removed adds up to approximately 84.231% of the original loadings. In social sciences, a cumulative Rotation Sums of Squared Loadings is seen as favorable when it exceeds 50%. The study reveals the discovery of 4 components through the application of Factor Analysis, showcasing

promising results. The rotated component matrix revealed that all items were retained in the study owing to the favorable factor loadings. The four factors under the study are –CET with 7 items, PMP with 5 items, TA with 5 items and CSE with 7 items.

**Model fit statistics**

The chi-square value split by the degrees of freedom (Chi-Square / df) is within the allowed range of 3, especially 2.189. The value of observed goodness of fit (0.898) above the suggested qualities. The RMR value obtained from the boundary estimation comes out to be 0.053. Within the scholarly community, the model in issue has drawn much praise; its fit criteria are sufficiently appropriate.

## RESULTS OF STRUCTURAL EQUATION MODEL

The reverse-scoring is used for the burnout scale, and this brings about remarkable findings: among IT people, spiritual quotient alone is able to reduce by 21% the total amount of "burnout" felt (p=0.000 B=0.208, b=0.155).This is the first study that has linked spiritual intelligence with burnout in the IT industry, and the results are not at all surprising. It shows that people who have higher spiritual intelligence—or in other words more of a spiritual intelligence quotient—can reduce by over 21% the total amount of burnout they feel. Spiritual intelligence equips people to adjust how they view their environment. In a negative work setting, they learn to cope with stress and challenges more effectively. By attaching themselves to a cause greater than themselves, people with high spiritual intelligence may also be surer of keeping an upbeat attitude and managing reasonably effectively any demands made on them at work. By facing up to the spiritual health of employees, this research may help to improve the mental and overall job satisfaction of such people. Further research and interventions aimed at such a holistic improvement of spiritual intelligence in the workplace might indeed reduce burnout rates and also lead to higher job satisfaction among IT people.

At the significance level of 0.000 spiritual intelligence impacts 12 %to the job satisfaction of IT Employees (B=0.117, b=0.327, p=0.000). Employees within IT who scored higher on spiritual intelligence were also more likely to rate themselves as being fully engaged in their work and report a higher sense of purpose and fulfillment with the work they are doing. Which in turn, can bring improvement to motivation, productivity and job performance in general. Employers should think about introducing workplace programs or practices that focus on spiritual health to promote employee satisfaction and well-being. In the end, what it means is that if people find meaning in work, a sense of satisfaction with the job will ultimately be heightened by spiritual intelligence, employees are able to access their source code for who they are, what they believe in and why we get up in the morning. Instead of simply giving them a career path or leadership position, work satisfies you from within. A more engaged and dedicated workforce will help the employees as well as the organization. In current fast-growing and mostly stress-full environments spiritual wellness might result a godsend to ensuring job contentment and overall wellbeing in IT workers.

## CONCLUSION

Spiritual intelligence impacted on Burnout among IT Employees it actually not effect, however researchers discovered that individual having high level of spiritual intelligence feels less stress and seems finding work more sense to enjoy. Spiritual practices like mindfulness, mediation, and maintaining a gratitude philosophy can be woven into the day to give IT employees the ability to go inward and outward more freely while traversing the waves of technology changes without riding them. Further, creating a culture that encourages support and feels inclusive of the importance of wellness for faculty can also serve to decrease burnout and enhance satisfaction in their work.

Spiritual intelligence affects IT Employees' job satisfaction. Studies have proved that the employees who have higher levels of spiritual intelligence have more possibilities to feel satisfied with the job and achieve better results. Such outcome can be explained by the reasons these employees have to be content with the job. Due to this nature, the employees are more likely to find a meaning they can enjoy and integrate into their work and perform their daily

tasks experiencing a certain level of resilience due to their ability to balance the existing stress and the feeling of inner peace they can feel. Overall, employers are likely to benefit from integrating spiritual intelligence training or practice in employee development with regard to job satisfaction gens.

The current study is a quantitative analysis of the impact of spiritual intelligence on job satisfaction and burnout. We recommend that future scholars conduct some qualitative research by interviewing IT employees. This qualitative approach may provide invaluable insights that supplement the quantitative findings of this study, and present a more complete picture of the subject. It also helps researchers to identify actual factors from all sorts within one universal environment where spirituality is said to have an impact which might or might not be having an effect on job satisfaction and burnout.Last but not least, interviewing employees helps researchers identify specific factors in a workplace environment that may contribute to the influence of spiritual intelligence on job satisfaction and burnout.Future research could also focus on the role of spiritual intelligence in other industries. Moreover, a study exploring potentially favorable consequences deriving from adding spiritual intelligence training to staff development plans could in point of fact provide organizations with useful reference information looking out for their own whole 'health and productivity.

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**Table 1. Estimates - Impact of SQ on Burnout and Job satisfaction**

| | | | Unstd Estimate | Std Estimate | P |
|---|---|---|---|---|---|
| Burnout | **<---** | **SQITProfessionals** | **0.208** | **0.155** | *** |
| Jobsats | **<---** | **SQITProfessionals** | **0.117** | **0.327** | *** |
| CET | <--- | SQITProfessionals | 0.819 | 0.92 | *** |
| PMP | <--- | SQITProfessionals | 0.896 | 0.801 | *** |
| CSE | <--- | SQITProfessionals | 0.952 | 0.846 | *** |
| TA | <--- | SQITProfessionals | 0.672 | 0.895 | *** |

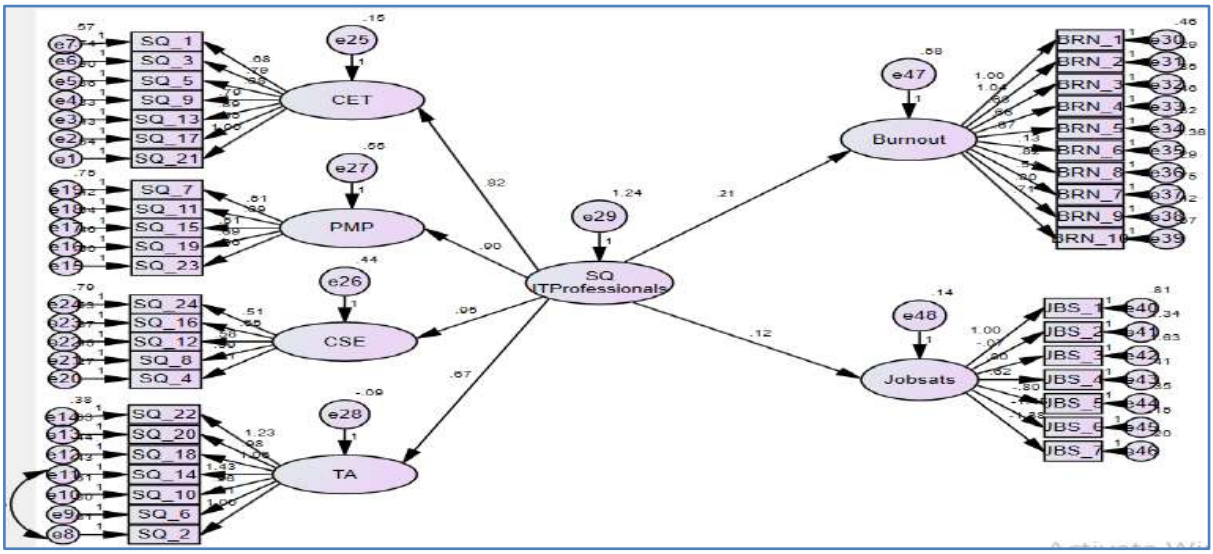


**Figure 1 Structural equation model – Impact of SQ on Burnout and Job satisfaction**

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**RESEARCH ARTICLE**

# The Influence of Virtual Technology on Production Systems: An Evaluation of Workflow Improvement and Cost Reduction

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## ABSTRACT

The effect of virtual technology on production systems is studied, with emphasis on its effect on workflow improvement and cost reduction. The efficiency and effectiveness of production processes are changed as industries more and more embrace virtual tools, such as simulation, digital twins and virtual reality. Using a quantitative approach, that is, quantitative data case studies and industry expert quantitative insights, this study evaluates the advantages and disadvantages of implementing virtual technology in production settings. This finding shows that virtual technology greatly improves workflow efficiency by simplifying operations and making real time decisions. What's more, organizations that utilize these technologies experience substantial cost savings, primarily reduced downtime, better resource allocation and higher quality products. The contribution of this paper is to help organizations to effectively adopt these innovations, using a framework for changing production systems through virtual technology.

**Keywords:** Virtual technology, Production Systems, Workflow Improvement, Cost Reduction, Manufacturing Environment.

## INTRODUCTION

A focus on the integration of virtual technology into production systems has risen as a crucial endeavor in organizations' operations in today's dynamic industrial environment for the objective of lowering costs and increasing operational efficiency. Virtual technologies such as simulation, digital twin and augmented as well as virtual reality enable an unparalleled visualization, analysis, and optimization of production processes. And as competition operates at a higher rhythm and markets begin to change, organizations are set to become innovative and be flexible in their production system so as to survive. The objective of this paper is to examine how virtual technology affects the production workflows and cost management. The research gauges the potential for a considerable lift in both productivity and profitability by working with how these technologies could cut operations expenses and encourage a more responsive manufacturing environment. Grounded in the empirical case study work and expert interviews, evaluation proposes a holistic picture of the consequences of adopting virtual technology in production systems. The most, this study hopes to provide industry stakeholders the knowledge and the tools for full utilization of virtual technologies in production workflows, to reduce the costs associated with production.

## REVIEW LITERATURE

**Production Systems with Virtual Technologies**

The types of technologies that comprise virtual technology are simulation, digital twins, AR/VR. M. K. Y. Lee et al. (2020) states that manufacturing can use simulation technologies to look at complex scenarios and simulate the changes without disrupting actual operations. The simulations can help to better make decisions by revealing process efficiencies and bottlenecks. The authors suggest targeted interventions, including inclusive recruitment and leadership development programs, to foster equitable workplaces and improve organizational performance (Xalxo, Seema, 2024).Additionally, the competitive landscape intensifies expectations for service quality, adding to workplace stress for employees. This highlights the need for effective stress management strategies in the sector (Karthikeyan et al.).

**Workflow Improvement**

It has been proven that implementing virtual technologies is very helpful for increasing workflow efficiency in a significant manner. J. S. T. According to Choi et al. (2022), AR applications help workers complete tasks faster by providing real time information and guidance, avoiding errors and increasing productivity. With this capability, employees can visualize complex data and processes to make informed decisions, which gets to streamlining workflows.The research emphasizes the need for stress management interventions at both individual and organizational levels to improve health, interpersonal relationships, and overall job performance in the healthcare sector (Nisa et al., 2024).

**Cost Reduction**

But cost reduction is clearly a first reason for adopting virtual technologies in production. According to A. G. H. C. Teo and E. T. Tan (2021), there are positive benefits in terms of operational cost reduction achieved from process optimization and less material waste after diffusion of simulation and digital twin technologies in organizations. The study reveals that, using virtual technologies, companies can spot inefficiencies and act to eliminate them proactively, from where the savings are huge.There is a significant correlations between these security measures and digital workplace effectiveness, providing valuable insights for policymakers to develop strategies that strengthen cyber security (Muthuswamy& Nithya, 2023).

**Challenges and Considerations**

Despite the clear benefits, implementation of virtual technologies in product development systems is not free of problems. The barriers mentioned by H. J. C. Wu et al. (2022) have high initial investment cost, lack of skilled personnel, and resistance to change within organizations. The successful implementation of virtual technologies is

hindered by these factors, and as such, need efficient change management strategies and training programs. Occupational exposure causes greater anxiety than community exposure, highlighting the need for workplace safety measures to mitigate fear and protect mental health. (Muthuswamy & Nithya, 2023)

## RESEARCH METHODOLOGY

**Research Design**

A quantitative research design is used for this study, and descriptive with Structured questionnaires are used to gather data which allows standardized, objective responses to help in the statistical analysis of Significance Difference between variables and ultimately provide the details on how virtual technology affects enhancing production efficiency.

**Data Collection Tools**

In this study, the impact of virtual technology on production systems is investigated. A convenience sample size of 100 participants were used. The data were summarized using frequency statistics to gain insights into usage patterns and trends. One-way ANOVA was employed to assess the significance of virtual technology's impact on workflow improvement and cost reduction across various demographic variables.

**MAJOR FINDING**

**NullHypothesis ($H_0$)**: There are no significant differences in Influence of Virtual Technologyamong different demographic Variables of the respondents.
**AlternativeHypothesis ($H_1$)**: There are no significant differences in Influence of Virtual Technologyamong different demographic Variables of the respondents.

The Above Table.1 examine the significant difference in Influence of Virtual Technology among different demographic Variables of the respondents.The analysis shows that gender does not have a significant effect on how virtual technology influences employees. With an F-Value 1.606 and a p-Value 0.078 (greater than 0.05), the null hypothesis is rejected, which means that the gender of the employees (male or female) does not significantly alter the perceived impact of virtual technology on workflow. This suggests that both male and female employees, irrespective of gender, experience similar levels of influence from virtual technology. The above analysis found a significant difference in the influence of virtual technology across different age groups. The F-Value 6.023 and the p-Value 0.012 (less than 0.05) indicate that age is an important factor in shaping how employees perceive the impact of virtual technology. Younger employees (such as those in the 25-30-year group) are likely to experience stronger effects, possibly due to greater exposure to or comfort with digital tools. Older employees, on the other hand, may not be as familiar with or receptive to the technology, resulting in a comparatively weaker perceived influence.

There is also a significant difference in how employees with varying educational backgrounds perceive virtual technology, with an F-Value 0.432 and a p-Value 0.045 (less than 0.05). Employees with higher educational qualifications, such as those holding a diploma or master's degree, tend to report a higher perceived impact of virtual technology, possibly due to their stronger technical and analytical skills. On the other hand, those with secondary or higher secondary education may not be as familiar with or confident in using advanced virtual tools, leading to a lower perceived influence.

The analysis shows that job role does not significantly influence how virtual technology is perceived, with an F-Value 1.933 and a p-Value 0.066 (greater than 0.05). This means that irrespective of an employee's job role whether they are engineers, technicians, production managers, or supervisor role there is no significant difference in how they experience the impact of virtual technology. The influence of virtual technology appears to be more dependent on other factors, such as familiarity or experience, rather than job role.

The data reveals that work experience plays a significant role in shaping how employees perceive the influence of virtual technology. With an F-Value 7.222 and a p-Value 0.016 (less than 0.05), the study indicates that employees with more years of work experience (11-25 years) report a stronger influence of virtual technology. This could be due to the increased exposure and adaptability that come with years of experience in the workforce, making them more likely to see the benefits of technology in improving workflow efficiency and reducing costs. Lastly, the analysis highlights that familiarity with virtual technology significantly impacts how employees perceive its influence. The F-Value4.256 and the p-Value 0.016 (less than 0.05) demonstrate that employees who are more familiar with virtual tools whether through prior experience or training are more likely to report a stronger influence of virtual technology. This indicates that the more an employee is exposed to virtual technology, the more they are likely to recognize and benefit from its advantages in improving workflow and reducing costs.

The findings from this study suggest that **age, education level, work experience,** and **familiarity with virtual technology** all significantly impact the perception of virtual technology's influence. Younger employees, those with higher educational levels, more work experience, and greater familiarity with virtual technology report a stronger perceived impact, indicating that these factors contribute to an individual's ability to adopt and utilize virtual technology effectively. On the other hand**, gender** and **job role** do not significantly affect the perceived influence of virtual technology, as shown by the non-significant p-values. The results highlight the importance of considering demographic factors like age, education, experience, and familiarity with technology when assessing how virtual technologies are perceived and adopted in organizations.

**RECOMMENDATIONS**

- Form cross-functional teams to collaborate on virtual technology projects, which can help integrate diverse perspectives and improve the overall effectiveness of the tools.
- Ensure robust IT support and cloud infrastructure for smooth integration of virtual technology, minimizing disruptions and optimizing performance.
- Continuously monitor the impact of virtual technology on workflow and cost reduction, using KPIs to assess its effectiveness and make data-driven adjustments.
- Create an environment that encourages innovation and experimentation with new technologies, where employees feel comfortable exploring virtual tools and solutions.
- Ensure that virtual technology adoption aligns with the organization's strategic objectives, particularly in terms of improving operational efficiency and reducing costs.
- Provide clear communication about the benefits of virtual technology, and involve key stakeholders in the decision-making process to reduce resistance and gain buy-in.

## CONCLUSIONS

In conclusion, the integration of virtual technology in production systems has proven to be beneficial, significantly enhancing workflow efficiency and contributing to cost reduction. The findings suggest that factors such as age, education, work experience, and familiarity with virtual technology play crucial roles in determining how employees perceive and adopt these technologies. While gender and job roles showed no significant impact, organizations can optimize adoption by targeting younger employees, providing specialized training for those with lower educational backgrounds, and fostering a culture of innovation and continuous learning. By implementing pilot programs, investing in strong IT infrastructure, and regularly evaluating the effectiveness of virtual tools, organizations can successfully leverage virtual technology to improve production processes and achieve long-term operational success

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**Table 1. Influence of Virtual Technologyamong different demographic Variables**

| Variables | Components | Mean | Std. Deviation | F-Value (df) p-value |
|---|---|---|---|---|
| **Gender Distribution** | Male | 3.50 | 0.525 | $F_{(2,97)}$ =1.606 p = 0.078 Significant |
| | Female | 4.00 | 0.708 | |
| **Age (In years)** | 25-30 | 4.75 | 0.273 | $F_{(5,94)}$= 6.023 p = 0.012 Significant |
| | 31-35 | 4.20 | 0.243 | |
| | 36-40 | 4.94 | 0.399 | |
| | **41-45** | 4.26 | 0.312 | |
| | 46-50 | 3.70 | 0.461 | |

| | | | | |
|---|---|---|---|---|
| **Education Level** | Diploma | 4.70 | 0.264 | $F_{(4,95)}$= 0.432<br>p = 0.045<br>Significant |
| | Master's Degree | 3.50 | 0.206 | |
| | Secondary | 4.26 | 0.312 | |
| | Higher Secondary | 3.70 | 0.461 | |
| **Job Role** | Engineer | 2.74 | 0.519 | $F_{(6,93)}$= 1.933<br>p = 0.066<br>Not Significant |
| | Technician | 2.90 | 0.587 | |
| | Production Manager | 3.79 | 0.655 | |
| | Supervisor | 4.00 | 0.872 | |
| | Project Coordinator | 4.10 | 0.845 | |
| | Quality Assurance | 4.02 | 0.733 | |
| **Work Experience** | 1-5 | 3.70 | 0.294 | $F_{(6,93)}$ = 7.222<br>p = 0.016<br>Significant |
| | 5-10 | 3.00 | 0.487 | |
| | 6-10 | 3.20 | 0.667 | |
| | 11-15 | 4.00 | 0.332 | |
| | 16-20 | 4.05 | 0.233 | |
| | 21-25 | 4.12 | 0.468 | |
| **Familiarity with Virtual Technology** | Low | 2.74 | 0.233 | $F_{(3,96)}$ = 4.256<br>p = 0.016<br>Significant |
| | Medium | 3.50 | 0.459 | |
| | High | 4.70 | 0.766 | |

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**REVIEW ARTICLE**

# Synbiotics: Emerging Themes, Current Developments and Future Trends A Review

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**ABSTRACT**

The primary goal of food is to provide enough nutrients and energy to support physiologic processes and general wellbeing. Every meal and feed has a purpose, and diet helps people consume bioactive chemicals. Synbiotics are significant bioactive elements of functional foods thathave a big impact on your health. These bioactive components offer one potential strategy for enhancing the perception of health and creating useful products. This review offers an overview of functional foods and the possible advantages of specific bioactive chemicals on human and animal health.

**Keywords:** probiotic; prebiotic; gut microbiota; gastrointestinal tract; bacteria; growth;

## INTRODUCTION

One of the greatest interfaces between the host, the environment, and the body's antigens is the human gastrointestinal (GI) tract. Around 60 tonnes of food and a profusion of environmentalmicrobes that pose a serious danger to gut integrity travel through the human GI tract in a typicallifetime (Elizabeth Thursby et al., 2017). The human digestive system's gut microbiota is incredibly varied and home to trillions ofmicroorganisms. Intestinal permeability, digestion, and metabolism, as well as immunological responses, can all be affected by changes in the composition and function of the gut microbiota. Alterations in the gut microbiota's balance result in a pro-inflammatory state that can induce a variety of diseases, from digestive and metabolic disorders to immunological and neuropsychiatricdisorders(Gomaa E.Z., 2020).

Probiotics are "live microorganisms" that, when given to a person in sufficient quantities, may have positive effects on their health (Vandenplas et al., 2013; Parvez et al., 2006). The term is from the Greek "for life" (Lilly D.M et al., 1965). The primary probiotics utilized globally are the gastrointestinal (GI) microflora species Lactobacillus and Bifidobacterium (Parvez et al., 2006; Underwood et al., 2009). Prebiotics are indigestible food components that may benefit the host by promoting the growth and/or activity of one or a few specific bacteria in the colon while also enhancing host health. Inulin and fructooligosaccharide (FOS), which are added to various foods as fat and sugar substitutes to improve texture or for their functional advantages, are the prebiotics that have received extensive investigation (Vandenplas et al., 2013; Gourbeyre et al., 2011). In the last few decades, a lot of study has been done on the health benefits that probiotics, prebiotics, and synbiotics confer. Functional foods are food supplements that have been shown tochange, adapt, and restore the body's natural gut flora (Pandey K.R, et al., 2015)..

**SYNBIOTICS CONCEPTS AND DEFINITIONS:**

Synbiotics refer to a blend of prebiotics and probiotics for the enhancement of human and animal health (Markowiak et al., 2017). More than 20 years ago, when Gibson and Roberfroid first introduced the idea of prebiotics, they also envisioned how pre- and probiotics might be joined to form synbiotics. At first, it appeared acceptable that a synbiotic might be described as a mix of a probiotic and a prebiotic, each component required to satisfy the requirements for either a probiotic or a prebiotic according to the prior scientific consensus definitions. Currently, the International Scientific Association for Probiotics and Prebiotics panel's consensus on what constitutes a synbiotic is as follows: "A mixture, comprising live microorganisms and substrate(s) selectively utilised by host microorganisms, that confers a health benefit on the host" (ISAPP, 2013). Synbiotics were developed to address potential issues with probiotics' ability to survive in the digestive tract since they possess both probiotic and prebiotic qualities (Rioux et al., 2005). By targeting the growth and/or activating the metabolism of one or a small number of health- promoting bacteria, a synbiotic product positively affects the host by enhancing the survival and implantation of live microbial dietary supplements in the gastrointestinal system (Cencic et al., 2010).This unique combination of a probiotic and prebiotic is presumably what gives synbiotics their health benefits (Scavuzzi et al., 2014).

Probiotics and prebiotics that have been chosen separately from one another and each contribute to a specific effect or health benefit are said to be complementary synbiotics. Synergistic synbiotics, on the other hand, also contain a probiotic strain and a prebiotic substrate, with the prebiotic being specifically designed to promote the growth of the cognate probiotic (S. Kolida etal., 2011).

**PROPERTIES AND FACTORS AFFECTING SYNBIOTICS**

The fundamental benefit of employing a synbiotic is that a probiotic does not do well in the digestive system without its prebiotic food. The probiotic will be more sensitive to oxygen, a low pH, and heat in the absence of its essential food source. The number of these beneficial bacteria isknown to remain stable because prebiotics offer an ideal setting for probiotics to thrive. Studies have shown that increasing the quantity of beneficial bacteria in our digestive systems significantly improves our health by utilising the synergistic effects of prebiotics and probiotics
Synbiotics function in two ways: i) by enhancing the probiotics' longevity and ii) by providing certain health advantages (Bhupinder Singh et al., 2010) .

When a synbiotic diet is ingested, the metabolic processes of the gut are modulated while the integrity of the gut's biostructure is preserved. Following the feeding period, studies showed a notable rise in short chain fatty acids, ketones, carbon disulfide, and methyl acetate levels, which suggested that the synbiotic diet may have health-promoting properties (Vitali. B et al., 2010).

**Symbiotic nutrition:**

One or more specially selected microbial strains may be present in probiotic products. The majorityof human probiotic bacteria are from the genera Lactobacillus, Bifidobacterium, and Lactococus, Streptococcus, and Enterococcus. Additionally, several yeast strains from the family Saccharomyces and some strains of Gram-positive bacteria from the species Bacillus are frequently found in probiotic products (Simon O, et al., 2005). Other titles for food components that are not digested in the gastrointestinal tract include prebiotic and dietary fibre. Prebiotics are fermented by narrowly specified groups of microbes, but dietary fibre is utilised by the majority of colonic microorganisms, which is a fundamental distinction between the two concepts (Ouwehand A, et al.,2005). Prebiotics can be divided into disaccharides, oligosaccharides (3–10 monomers), and polysaccharides depending on how many monomers are bonded together. The oligosaccharides fructooligosaccharides (FOS), galactooligosaccharides (GOS), isomaltooligosaccharides (IMO), xylooligosaccharides (XOS), transgalactooligosaccharides (TOS), and soybean oligosaccharides (SBOS) are among the most promising and fulfilling prebiotic substances, according to in vitro and in vivo studies (Patterson J et al., 2003; Annison G et al., 2003). Synbiotics are used to promote the growth of particular native bacterial strains that are already present in the gastrointestinal system as well as to boost the survival of helpful microorganisms that are added to food or feed (Gourbeyre P et al., 2011). Synbiotic products with bacteria from the Bifidobacterium or Lactobacillus species and fructooligosaccharides appear to be among the most popular (Markowiak, P, et al., 2017).

**Natural Sources of Synbiotics**

Probiotics can be obtained from natural sources such as yoghurt, cultured buttermilk, and cheese. Japanese miso, tempeh, sauerkraut, beer, sourdough bread, kimchi, olives, and pickles are some of the various foods made by bacterial fermentation. Kefir is yet another fermented dairy item. In non-dairy fermented substrates, such as soy-based products, grains, legumes, cabbage, maize, pearl millet, and sorghum, beneficial microbes are also found (Syngai GG et al., 2015; Anandharaj M et al., 2014; Kechagia M et al., 2013). Probiotic bacteria are typically made available in the gut by the ingestion of dairy-based fermented foods, which are typically made using milk as the initial raw material. Kefir, fermented milk, andnatural yoghurt smoothies are some of the most widely consumed fermented beverage items on the market today. Dairy products typically contain probiotic cultures, and customers are accustomed to milk-based goods containing bacteria (Terpou A et al., 2018; Kandylis P et al., 2016).

Vegetables, maize, legumes, and fruits are just a few of the basic ingredients that can be used to make probiotic beverages. Probiotic cultures have been tried with juices from a range of fruits as an alternate nutrient source. Fruit juices also serve as a supply of carbohydrates, vitamins, and minerals for the development of probiotic strains. The advantage of drinking probiotic beverages over fermented solid foods is that liquid diets flow through the body more quickly without spending as much time in the stomach's acidic environment, which is beneficial for the higher viability of probiotic strains to reach the intestine. (Chavan M et al., 2018; Saeed M et al., 2018; Dahiya D et al., 2022). Fruits like pineapple, blackberries, apples, strawberries, lemons, mangoes, grapes, cashews, oranges, carrots, and beets are used to make probiotic products with a fruit basis (Panghal A et al.,2018).

Apples, guavas, bananas, and melons, among other fruits, contain pectins and fruit fibres that maywork as probiotic bacterium carriers and prebiotics by acting as strong adhesive supports for the bacteria ( Nagpal R et al., 2012).
There is enough data to conclude that the eight types of prebiotic dietary fibre listed below help people maintain good digestive health. Included in this group are beta glucans, fructo-oligosaccharides (FOSs), oligofructose, inulin, galacto-oligosaccharides (GOSs),isomaltooligosaccharides, guar gum, lactulose, resistant starch, maltodextrin, xylooligosaccharides, and arabinooligosaccharides (Carlson J L et al., 2018; Moshfegh AJ et al., 1999). Leeks, asparagus, onions, wheat, garlic, chicory, oats, soybeans, and Jerusalem artichokes are all naturally high in inulin, a prebiotic. The estimated daily intake of naturally occurring prebiotics [inulin and FOSs] in the US and Europe is several grams per day (Carlson J L et al., 2018; Van Loo J et al., 1995).

Pulses frequently include shorter chain GOS, including raffinose, stachyose, and verbascose. Fruit and milk beverages, dairy products, and isolated GOS can all be combined. Some newborn formulaand baby foods also contain GOS and FOS combinations since research suggests they are effective at lowering the likelihood of allergies. Fruit, vegetable, and cereal-based foods include FOS, whichcan be produced by either enzymatically degrading inulin or transfructosylating sucrose (Osborn et al., 2013). The non-digestible carbohydrates known as fructans [including fructo-oligosaccharides (FOS) and inulin] and galactans [including galacto-oligosaccharides (GOS)] are widely accepted as prebiotics(Gibson et al., 2017). Galactans include -GOS (found in plant foods like pulses) and -GOS, which can be produced enzymatically from lactose. Isolated prebiotics can be used as functional food ingredients to help product reformulation (for example, by replacing fat and sugar). Some fruits, vegetables, and cereal-based foods include fructans (Dahiya D et al., 2022).

**Synbiotics and Applications in Health*:***

In the past ten years, research on probiotics and prebiotics combined as synbiotics to improve human and animal health has increased, including numerous clinical trials that have evaluated a wide variety of synbiotic formulations (Janina A et al., 2018). In recent years, the makeup and health of the gut microbiota have attracted a lot of study attention.Studies have examined the benefits of therapies for a variety of systemic diseases affecting the gastrointestinal, cardiovascular, neurological, inflammatory, oncological, and endocrine systems (Oniszczuk A, et al., 2021). Synbiotics have been shown to be helpful in treating a number of conditions related to gut health, including metabolic syndrome, decreased risk of colon cancer, decreased colonic tissue cell proliferation, prevention and treatment of irritable bowel syndrome and irritable bowel disease, improved mucosal structure, reduced lactose intolerance, and improved epithelial barrier function.With regard to the gut-brain axis, it aids weight loss, treats autism, and lessens stress, anxiety, anddepression. Evidence suggests that synbiotics also manage food allergies, have antioxidant properties, lower the risk of heart disease, prevent biliary disease, regulate blood lipid levels, treat osteoporosis, hypocholesterolemia, and hepatic encephalopathy, as well as suppress NK cells in Peyer's patches(Krumbeck J et al., 2018).

**Commercial synbiotics: obstacles, challenges and futureprospects:**

According to the Synbiotics Market: Global Industry Analysis 2022-2030, the market for synbiotics was assessed at USD 0.80 billion in 2022 and increased at an 8.20% CAGR between 2022 and 2030. By 2030, the market is anticipated to reach USD 1.50 billion. Prebiotics and probiotics are increasingly needed in functional foods and beverages. Over the forecast years, it is expected that growing consumer health consciousness and the demand for nutritional supplements will considerably drive synbiotics market growth. To provide nutrient enrichment and further associated health benefits, manufacturers in the food and beverage industry and other sectors are promoting functional ingredients like prebiotics, probiotics, and synbiotics in their product offerings. Similar to this, it is anticipated that the use of synbiotics in the pet food and pharmaceutical industries, as well as the growing significance of fibres in the food and beverage sector, will increase demand and strengthen the synbiotics market's supply chain. Future demand for synbiotic products is also expected to be driven by a shift in consumer preference towards health supplements as a result of changing food and lifestyle patterns. Van den Nieuwboer et al., 2016 said that although there are market prospects, there are a numberof technological and scientific obstacles. The biggest obstacle was specifically the difficulty in demonstrating clinical efficacy. Consumers frequently receive synbiotic products in the form of food, including a variety of cultured dairy and nondairy products. Prebiotics can be employed in most food applications, whileprobiotics and synbiotics may not be as feasible in many non-refrigerated foods due to their environmental sensitivity (Saulnier et al., 2008; Janina A et al., 2018).

New microencapsulation methods, however, that shield the bacteria from potentially harmful processing procedures could result in a wide range of new synbiotic products ( Konar et al. 2016). To confirm additive or synergistic effects, probiotic-only and prebiotic-only controls may also berequired in addition to standard placebo controls. It is important to explain the rationale behind theprobiotic and prebiotic choices. Multiple prerequisites must be met in order for synbiotic combinations to be developed that are therapeutically successful. Identifying

minimum effective doses of each component is often expected, as was mentioned before. It can be difficult to include suitable controls in synbiotic experiments ( Janina A et al., 2018 ).

## CONCLUSION

It is not unexpected that a wide range of diseases and dysfunctions have been linked to an imbalance in either composition, numbers, or habitat of the gut microbiota given that it appears tocontribute to practically every aspect of the host's growth and development. Since instruments forsynbiotic study are now available, the future will be demanding but also rewarding. We have already made significant progress in our understanding of synbiotics and how they work. Thus, the study of synbiotics may eventually lead to the development of a new scientific subject, practiceand research creating a new path for personalised healthcare and perhaps even upcoming biotherapies. In order to give the benefits of synbiotics, commercial preparations used in the healthmarket are manufactured using specific strains of probiotic microorganisms, and coatings of capsules or tablets are constructed of various plant-sourced materials. Each preparation is created utilising a unique combination of strains and plant components to provide remedies for particularhealth-related problems. Given the paucity of studies to date, this definitely calls for more study.

**Conflict of Interest**
The authors declare that there is no conflict of interest associated with this article.

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Ms. Lekha A Reddy went through the peer-reviewed journals, databases, drafted the article, designed the tables and figures. Ms. T. H. Hema ( supervisor ), was involved in planning andsupervising the work.

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**(Table 1: Summary of the common Probiotics and Prebiotics used in human nutrition )**

| PROBIOTICS |
|---|
| **1. Type *Lactobacillus*** |
| (*L. acidophilus*<br>*L. amylovorus*<br>*L. casei*<br>*L. gasseri*<br>*L. helveticus*<br>*L. johnsonii*<br>*L. pentosus*<br>*L. plantarum*<br>*L. reuteri*<br>*L. rhamnosus* |
| **2. Type *Bifidobacterium*** |
| *B. adolescentis*<br>*B. animalis*<br>*B. bifidum*<br>*B. breve*<br>*B. infantis*<br>*B. longum* |
| **3. Other Lactic Acid Bacteria** |
| *Enterococcus faecium*<br>*Lactococcus lactis*<br>*Streptococcus thermophilus* |
| **4. Other Microorganisms** |
| *Bacillus clausii*<br>*Escherichia coli Nissle*<br>*Saccharomyces cerevisiae (boulardi)* |

| PREBIOTICS |
|---|
| FOS<br>GOS<br>Inulin<br>XOS<br>Lactitol<br>Lactosucrose<br>Lactulose<br>Soy oligosaccharides<br>TOS |
| Abbreviations:<br>FOS—fructooligosaccharides;<br>GOS—galactooligosaccharides;<br>TOS—transgalactooligosaccharides;<br>XOS—xylooligosaccharides. |

**(Source: *European Food Safety Authority (EFSA), 2017 ; Crittenden R et al., 2009*)**

**(Table 2: Summary of the common synbiotics used in human nutrition )**

| |
|---|
| *Lactobacillus* genus bacteria + inulin |
| *Lactobacillus*, *Streptococcus* and *Bifidobacterium* genus bacteria + FOS |
| *Lactobacillus*, *Bifidobacterium*, *Enterococcus* genus bacteria + FOS |
| *Lactobacillus* and *Bifidobacterium* genus bacteria + oligofructose |
| *Lactobacillus* and *Bifidobacterium* genus bacteria + inulin |

***(Source: Paulina M, et al., 2017)***

**Table 3: VARIOUS FOOD SOURCES OF PREBIOTICS, PROBIOTICS AND SYNBIOTICS**

| PROBIOTIC FOODS | PREBIOTIC FOODS | SYNBIOTIC FOODS |
|---|---|---|
| 1. FERMENTED SOY FOODS:<br>i) Tempeh<br>ii) Miso<br>iii) Natto | 1. ASPARAGUS | 1. BANANA SMOOTHIE WITH KEFIR OR YOGURT |
| 2. KEFIR | 2. BANANA | 2. STIR FRY MADE WITH TEMPEH, ASPARAGUS, GARLIC AND LEEKS |
| 3. KIMCHI | 3. CHICORY | 3. YOGURT WITH BLUEBERRIES |
| 4. KOMBUCHA | 4. GARLIC | 4. OATS WITH KIMCHI/ SAURKRAUT |
| 5. SAUERKRAUT | 5. JERUSALEM ARTICHOKES | 5. MISO SOUP WITH GARLIC, ASPARAGUS AND LEEKS |
| 6. YOGURT | 6. LEEKS | 6. CHICORY AND ARTICHOKES SALAD WITH NATTO |
| 7. CULTURED BUTTERMILK | 7. OATS | 7. SPICY BUTTERMILK WITH LEEKS AND GARLIC |
| 8. CHEESE | 8. ONIONS | 8. STUFFED CHEESE WITH LEEKS, ASPARAGUS AND GARLIC |
| | 9. SOYBEANS | |

***( Source: Carlson J L et al., 2018 )***

**UNIQUE FEATURES OF FUNCTIONAL FOODS:**

- being a conventional or everyday food;
- to be consumed as part of the normal/usual diet;
- composed of naturally occurring (as opposed to synthetic) components perhaps in unnatural concentration or present in foods that would not normally supply them;
- having a positive effect on target function(s) beyond nutritive value/basic nutrition;
- may enhance well-being and health and/or reduce the risk of disease or provide health benefits so as to improve the quality of life including physical, psychological and behavioural performances;
- have authorized and scientifically based claims

**( Source: Gibson & Williams, 2005 )**

**PROBIOTICS:**

- It should have a demonstrable beneficial effect on the host,
- It should be non-pathogenic in nature,
- It should be non-toxic,
- It shouldn't have any adverse effects on humans and animals,
- It should be able to survive in the gastro-intestinal tract environment,
- It should retain the stability during the intended shelf life of the product,
- It should contain adequate number of viable cells to confer the health benefit,
- It should be compatible with product storage containers to retain the stability

**(Source: Indu et al., 2001 ; Harish et al., 2006 )**

**PREBIOTICS:**

- Ability of micro-flora modulation.
- Selective metabolic action/ stimulation of growth of probiotics.
- Stability in various processing conditions.
- Fermentation by intestinal microbiota.
- Easy storage.
- Resistance to digestion in the upper parts of the alimentary canal.
- Beneficial effect on host's health.

**(Source: Pandey, K. R. et al 2015 ; Swennen K. et al 2006)**

**(Figure 1: Summary of the properties and characteristics of Probiotics and Prebiotics)**

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**RESEARCH ARTICLE**

# An Economic Study on Rural Household Energy Consumption in Theni District

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**ABSTRACT**

Energy is the unit of measurement for all types of effort, and its use has been rising globally. It is become a necessary component of modern life. The energy is one of the fundamental factors in the functioning of any civilized society needed to improve better life style and socio- economic development of the Nation. Energy generally has been simply defined as "the ability or capacity to do work". Planned industrialisation, economic development, modernisation of agriculture, population growth, and rise in the human standard of living has created a burgeoning demand for energy in India. Hence the energy consumption significance role of cooking and lighting but its consumption varies from rural to urban areas. Rural people used fire, wood, agricultural residues, dung, leaves, kerosene, gas and electricity where as urban people depend mostly on kerosene and electricity. Hence It cannot be denied that consumption pattern of energy depends on socio- economic background of the householders. But to save time and avoid smoke, rural residents would rather have gas and electricity than firewood, manure and agricultural waste.

**Keywords:** Energy Household, Consumption, Electricity, Kerosene and LPG.

## INTRODUCTION

The word "Energy" is derived from the Greek word 'Energeia,' which means's in work. It is the power or capacity to do work. Energy has become an important and one of the basic infrastructures for human development and

economic growth of a country. Since energy may be used to measure any type of effort, its consumption has been rising globally. It is now a fundamental necessity in today's world. The energy is one of the fundamental factors in the functioning of any civilized society needed to improve better life style and socio- economic development of the Nation. Energy generally has been simply defined as "the ability or capacity to do work". Planned industrialisation, economic development, modernisation of agriculture, population growth, and rise in the human standard of living has created a burgeoning demand for energy in India. Hence the energy consumption significance role of cooking and lighting but its consumption varies from rural to urban areas. Rural people used fire, wood, agricultural residues, dung, leaves, kerosene, gas and electricity where as urban people depend mostly on kerosene and electricity. It is seen that there is a remarkable changes in the rural energy consumption in recent years. The people used dry leaves, plants, and fire wood and agricultural residues about three decades ago. But majority people use gas and electricity for preparing tea, meals, and heating water in recent times.

## REVIEW OF LITERATURE

Dinesh Kumar Shahi et al. (2019) have been analysed the household energy demand has increased significantly in various countries including Nepal. In the case of Nepal, 94 per cent of energy use is in the domestic sector. Even though there is a chance that electricity production may skyrocket, load shedding persists because of the enormous demand for electricity. The usage of electricity has a significant impact on a country's development and quality of life. Therefore is not a sufficient number of researches done about electricity consumption in different climate regions of Nepal which are analysed by the income level of residents. This study employs electrical appliances in homes to examine the rate of power use and provides descriptive data on household energy use trends. Karabi Biswas et al. (2020) in this work the author reveals that energy plays a very important role in the development in any region or country. Energy fulfils the fundamental requirement of subsistence living in the form of cooking and lighting apart from assisting in the productive processes like transport, agriculture and industry. In terms of global energy consumption from all sources, India comes in third position, trailing only China and Japan. Given that it accounts for half of the nation's overall energy consumption, the residential sector is one of the major energy consumers.

**Important of the Study**

The energy consumption is one of the basic necessities of human life. In fact, human life is unthinkable in absence of energy people used by the different sources of energy for cooking food and heating water. Lack of firewood cost of gas, and Kerosene Lake of time for cooking and socio- economic background have changed the energy consumption in rural in Theni District.

**Objectives of the Study:**

The specific objectives of the present study are:

1. To study the changes scenario of rural energy consumption.
2. To identify the causes of changes in the energy consumption in rural area in Theni District.
3. To find out the problems of rural energy in Theni District.

## METHODOLOGY

The present study was conducted in the rural areas of Theni District of Tamil Nadu. It consists of Five Taluks, namely Andipatti, Bodinaykanur, Periyakulam, Theni and Uthamapalayam Taluks. The study gave equal importance of the five Taluks irrespective of size of population, size of age and environment thus; thirty samples have been selected from each taluk. The total number of respondents is 150. The sample random sampling method was used for data collection. The data collected from the both primary and secondary data and analyzed with appropriate statistical tools have been used.

**Rural Energy Consumption in India**

India is the second popular country in the world after China and has extremes of ecological diversity. 90 per cent of the population in India still live in the rural areas: and meeting their energy requirements in a sustainable manner continues to be a major challenge for the country.

**House Hold Rural Energy Consumption**

The house hold sector is one of the major components of energy consumption. The national budget accounts it for about 50 per cent of energy consumption in India. It is seen that about 75 per cent population lives in rural areas. Rural people needs energy for cooking, lighting, and water heating and supply since the present study is concerned with households. It analysis the energy consumption for cooking and water heating in rural areas of Theni District. The demand for energy is mint by both the commercial and non- commercial energy. Commercial energy includes LPG. Kerosene, Electricity and Firewood, on the other hand non - commercial energy refers to dry leaves, sticks and plants, agricultural residues and dung mainly obtained freely from own or neighbours farms.

**Changing Energy Consumption**

People live in rural areas have been changing energy consumption and water heating for the last three decades in Theni District. It could be observed from the Table: 1. The changing energy consumption in rural areas for the last 34 years. It is seen that the number of fire wood users decreased from 68.67 per cent in 1990 to 32 per cent in 2024. The number of agricultural residues users decreased from 14. per cent in 1990 to 6 per cent in 2024. The number of respondents who use dung as energy decreases from 9.33 per cent in 1990 to 3.33 per cent in 2024. The number of kerosene user's decreased from 15.33 per cent in 2000 to 8.67 percent in 2024. It is important to note the number of respondents who use LPG increased from 0.67 per cent in 2000to 42 per cent in 2024.The number of respondents who utilize electricity increased from 2.66 per cent in 2010 to 8 per cent in 2024 the study observes that people shift from non- commercial energy to commercial one and they use almost all types of energy in rural areas. The study also found that number of respondents who use gas (42 per cent) is more than fire wood (32.0 per cent) users in the study area. It shows the changing energy consumption in rural areas in current years.

**Reasons for Changing in Energy Consumption**

The reasons for energy consumption changes in different place to place time to time and family to family, this is because the availability of energy sources, time for cooking cost of energy and socio- economic back ground play need role in the use of energy in rural area in Theni District. It could be seen from Table -2 the study shows that among the total households (150) 21.33 per cent change the energy consumption due to their socio – economic back ground and style. In fact, education income and nature of occupation have modified their standard of living and these have great impact of energy consumption. Among the total households 20.67 per cent households 6 per cent and 3.33 per cent considers lack of agricultural residues and dung as the causes of change in energy consumption in rural areas in Theni District.

The study identify that about 16 per cent households change the energy due to lack of time. It is seen that 20 per cent households state that availability of commercial energy such as LPG and electricity bring about change in energy use of study area. More over 12.67 per cent households change energy due to change in cropping pattern and land used for non- agricultural purpose. They convey that these change affect; the availability of agricultural resides and fire wood. Because of this, their home needs are met by LPG and electricity. Thus the study found that change in life style. Cropping pattern, lack of time for cooking, lack of fire wood, dung and agricultural residues change energy consumption in rural areas in Theni District.

**Various Problems of Energy Consumption**

The people faced by the various problems in energy consumption in rural areas in Theni district. Lack of energy, cost of energy changing environment, delay in the supply of LPG, lack of energy subsidy, lack of knowledge about modern energy and accident are some problems identified in the study area.

Table -3 reveals that various problems of respondent in energy consumption in rural areas in Theni district. The study depicts that out of the total respondent 16 per cent suffer from lack of energy like fire wood and agricultural residue. about 20.67 per cent respondents suffers from increase in the cost of fuel like fire wood, LPG and electricity. The changing in environment such as cropping pattern, land used for agricultural purpose, extension of urbanization and changing life style reduce the availability of non- commercial energy and increases price of commercial energy. In fact, it causes for the many problems of energy consumption in the study area. Moreover, delay in the supply of LPG and frequent power cut create the problems in energy use pattern. The reveals that 25.33 per cent respondents suffer from this problem. People in rural areas anticipate subsidies for using energy sources like electricity and LPG. The non – availability of energy subsidy affects 12 per cent respondents. The study observed that 6.67 per cent respondents do not know how to use LPG gas and they fear about it. Moreover, 4 per cent respondents state that use of energy like LPG and electricity causes accidents.

## CONCLUSION

The study find out the changes in energy consumption, its causes and problems in rural in Theni district. Energy is the universal measurement of all kinds of work and its consumption has been increasing worldwide. It has become a basic requirement of modern life. In fact, it is the backbone of present day civilization. Thus, with an increase in the living standard of human beings, the energy consumption is also accelerated. There is a strong correlation between standard of living and energy consumption. Hence It cannot be denied that consumption pattern of energy depends on socio- economic background of the householders. But to save time and avoid smoke, rural residents would rather have gas and electricity than firewood, manure and agricultural waste. The study observes that the number of households using gas increased from 0.67 per cent to 42 per cent in 2024. On the other hand the number of householders using fire wood decreases from 68.67 per cent in 1990 to 32 per cent in 2024. The study observes that convenience of people influence more than availability in the determination of energy consumption in rural areas in Theni district.

### Suggestions

1. Energy subsidy may provide for domestic use at least based on income of the uses.
2. Awareness should be developed among the rural mass about how to use LPG and shun accident and fear.
3. To lessen the scarcity of a certain energy source, people can use electricity, gas, firewood, or kerosene.
4. Women without jobs might utilise firewood, plants, and dried leaves to reduce their reliance on LPG and electricity.
5. Gas and electric stove and burners should be handled carefully and switch off when not in use. Moreover, children should be advised to avoid mishaps.

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**Table – 1 : Changing Energy Consumption**

| Year | Fire wood | Agricultural residue | Dung | Kerosene | Gas | Electricity | Total & percentage |
|---|---|---|---|---|---|---|---|
| 1990 | 103 (68.67) | 21 (14.0) | 14 (9.33) | 12 (8.0) | - | - | 150 (100) |
| 2000 | 93 (62.0) | 20 (13.33) | 13 (8.67) | 23 (15.33) | 1 (0.67) | - | 150 (100) |
| 2010 | 69 (46.0) | 15 (10.0) | 9 (6.0) | 16 (10.67) | 37 (24.67) | 4 (2.66) | 150 (100) |
| 2020 | 53 (35.33) | 10 (6.67) | 6 (4.0) | 14 (9.33) | 58 (38.67) | 9 (6.0) | 150 (100) |
| 2024 | 48 (32.0) | 9 (6.0) | 5 (3.33) | 13 (8.67) | 63 (42.0) | 12 (8.0) | 150 (100) |

**Source: Compiled from the Field study.**

**Table- 2 : Reasons for Changing in Energy Consumption**

| Sl. No. | Cause | No. Of Sample Households | Percentage |
|---|---|---|---|
| 1 | Lack of fire wood | 31 | 20.67 |
| 2 | Lack of Agricultural resides | 9 | 6.0 |
| 3 | Lack of Dung | 5 | 3.33 |
| 4 | Lack of Time | 24 | 16.0 |
| 5 | Availability of commercial energy | 30 | 20.0 |
| 6 | Changing life style | 32 | 21.33 |
| 7 | Cropping pattern | 19 | 12.67 |
| **Total** | | **150** | **100** |

**Source: Compiled from the Field study.**

**Table -3 : Various Problems of Energy Consumtption**

| S.No | Problem | No. of Respondents | Percentage |
|---|---|---|---|
| 1 | Lack of energy | 24 | 16.0 |
| 2 | Cost of fuel | 31 | 20.67 |
| 3 | Changing Environment | 23 | 15.33 |
| 4 | Delay in supply | 38 | 25.33 |
| 5 | Lack of subsidy | 18 | 12.0 |
| 6 | Lack of knowledge | 10 | 6.67 |
| 7 | Accident | 6 | 4.0 |
| **Total** | | **150** | **100** |

**Sources: Compiled from the Field study**

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**CASE STUDY ARTICLE**

# Management of Subclinical Hyperthyroidism through Ayurveda: Case Report

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## ABSTRACT

Hyperthyroidism or thyrotoxicosis refers to an overactive thyroid gland. Subclinical hyperthyroidism (also known as subclinical overactive thyroid gland or mild or severe subclinical hyperthyroidism) is diagnosed when thyroid function tests show normal thyroid hormone levels of FT3 and FT4 but low or suppressed levels of thyroid-stimulating hormone TSH). Occasionally the FT3 levels may be high. Mild subclinical hyperthyroidism is more common than severe subclinical hyperthyroidism. Subclinical hyperthyroidism can have an exogenous (external) cause or endogenous (internal) cause. Exogenous causes are more common i.e. taking too much levothyroxine or caused by the drug amiodarone. Endogenous causes may include Graves' disease, toxic multinodular goitre, toxic adenoma and certain types of thyroiditis. Most people who suffer from subclinical hyperthyroidism don't present with any of the symptoms of hyperthyroidism (an overactive thyroid). However, in some cases, symptoms are similar to that of hyperthyroidism. A 34-year-old female patient came to Panchakarma OPD with complaints of weight loss anxiety, palpitation, tremors and hair fall. A treatment protocol was planned based on the signs and symptoms. *Snehapana* with *Mahatiktakghrita* followed by *Virechana* and *Sansarjana karma*. After the *Shodhana, Shamanayoga* of *Vidarikandhachurna, Shatavari churna* and *Brahmi vati* was given to the patient for two months. There is a marked improvement in the bio-chemical parameters and in the symptoms of subclinical hyperthyroidism.

**Keywords:** Subclinical Hyperthyroidism, *Snehapana*, Virechana, *Vidarikandhachurna*, *Shatavari churna* and *Brahmi vati*

# INTRODUCTION

In India more than 5 million cases per year are diagnosed with Hyperthyroidism. Hyperthyroidism can accelerate body's metabolism significantly causing sudden weight loss, a rapid or irregular heartbeat, sweating & nervousness and irritability. Hyperthyroidism is more prevalent in women than in man. Subclinical hyperthyroidism is a biochemical diagnosis characterized by a decreased serum thyroid-stimulating hormone (TSH) and normal serum thyroxine (T4) and triiodothyronine (T3)concentrations. Proposed grading systems distinguish between mild (TSH, 0.1-0.4 mIU/L) and severe Subclinical hyperthyroidism (TSH, <0.1 mIU/L).[1] Thyroid Hormone is required for the normal functioning of numerous tissues in the body. In health the thyroid gland predominantly secrets thyroxine (T4) which is converted in to triiodothyronine (T3) in other organs by the selenium dependent enzyme iodothyronine deiodinase. T3 blinds to the thyroid hormone receptor in the nucleus of the cell. The thyroid gland is the only source of thyroid hormone in the body, the process requires iodine and the amino acid tyrosine. Iodine in the bloodstream is taken by the gland and in corporate in to thyroglobulin molecules. The process is controlled by the TSH which is secreted by the pituitary gland. Not enough iodine or not enough TSH can result in decreased production of thyroid hormones. The Hypothalamic – Pituitary – Thyroid axis plays a key role in maintaining thyroid hormone levels within normal limits. The production of TSH by the anterior pituitary gland is stimulated in turn by thyrotropin releasing hormone (TRH) released from hypothalamus. Fundamental effect is deficient production of thyroid hormones due to various factors, but most common for is dietary lack of iodine. Which gets result in lack of thyroxin. Thyroxin is made up of by iodine and tyrosine (Amino acid). Deficient thyroid hormone production causes excessive TSH Stimulation which lead to hyperplasia of follicular epithelium as well as formation of new thyroid follicles. Rapid and prolonged changes of hyperplasia result in continued growth of thyroid tissue while involved areas undergo fibrosis nodular goitre. Thyrotoxicosis is a toxic condition of tissues exposed to the excessive thyroid hormones. Thyroid hormones metabolism runs in hypothalamus – pituitary – thyroid axis, but target tissues of thyroid dysfunction are multiple.[2]

**Case report**

A 34-year-old female patient, housewife by profession, who was apparently well one year back, developed progressive hair fall with itchy scaly scalp, palpitation and fatigue initially. Then she gradually developed tachycardia, tremors and breathlessness doing daily routine work. These symptoms were followed by anxiety, weight loss. She was diagnosed as Subclinical Hyperthyroidism followed by blood test (Thyroid profile) in which T3, T4 values are elevated and depressed TSH levels. Patient was put on Tab. Neo-Mercazole 10 mg/day (Carbimazole, anti-thyroid medication) and Tab. BetacapTR 40mg (propranolol, beta blocker). While being on medication, she developed some associated complaints like insomnia, itchy papule over planter surface of feet and irregular bowel habits and itching sensation in eyes. Radio iodine uptake to rule out malignancy. Radio iodine study detected both lobes of thyroid gland show decreased tracer uptake and scan finding suggestive of Thyroiditis. But even after taking medicine she didn't get much relief. As, Thyroid Stimulating Hormone (TSH) levels were not coming into physiological range, he stopped the medication against medical advice four months before his first visit to OPD in December 2023. Symptoms like heavy hair fall with dandruff, tremors, anxiety, increased appetite along with decreased weight and breathlessness and scanty irregular mensuration were the chief complaints during his visit to the OPD. On examination there was mild swelling of thyroid gland, dryness of skin and palpitation, heart rate was 112/min. and blood pressure was 98/60 mm of Hg.

Patient was provisionally diagnosed Bhasmaka Roga as *Kapha avritavata (vata* obstructed by *Kapha)* with *Pittaanubandha* (associated with *Pitta)* based on the symptoms like *Dourbalya* (tiredness), *Galapaka* (inflammation of thyroid gland), *Atyagni* (excessive appetite) can be attributed to *Pittanubandhatwa*. On evaluation it is found that the symptoms of *Pitta vriddhi, Vata vriddhi* and *Kapha kshaya* are more observed in hyperthyroidism. Further *Kapha kshaya* induces *kshaya* of *Rasa*, *Mamsa, Meda, Majja* and *Sukra Dhatus, Artava*is a *Updhatu* of *Rasa dhatu* so it also found to be decreased due to *Rasa kshaya* caused by *Kapha kshaya*. In this disease all the *Dhatus* become debilitated

and person becomes cachectic and suffers from syndrome of *Hritpeeda, Hriddrava, Kampa, Shosha, Alpayapicheshtaya Sharma, Sphikgreevodar Sushkata, Krishata, Shrama, Kesha-Nakha Prapatanam, Dourbalya, Asthi Saushirya, Ashaktimaithune, Yathochitkala-darshanam and Alpa Artavata*.[3]. The *Shamana* (palliative medicines) and *Shodhana, Virechana* (therapeutic purgation) was planned. Treatment schedule followed is enlisted at Table 1.

**Observations**

Thyroid profile before and after (TSH, free T3 and T4) is mentioned in Table 2 along with laboratory reports. Patient mentioned increased appetite and normal bowel movements after *Deepana* and *Pachana* and dryness of skin and scalp, insomnia was reduced during *Snehapana* and *Abhyanga* and Swedana, palpitations and watery papule were completely alleviated after *Virechana*. After administration of *Shamana* drug; major relief in tremors and breathlessness were observed. Blood parameters were improved approaching towards normal value after *Shamana chikitsa*.

## DISCUSSION

*Virechana* is the most preferred *shodana* in hyperthyroidism. As it corrects pitta related pathologies which are seen in hyperthyroidism. It does *vatanulomana.* Periodically ad ministered *virechana* delays the *dhatupaka* there by preventing further tissue destruction. As *Virechanadravya* posseses *ushna, tikshna, sukshma, vyavayi and vikasi* properties, by virtue of its *virya* the *Virechanadravya* reaches *hridaya*. *Hridaya* here can be considered as nerve plexus in brain where it may help in balancing the hypo- thalamo pituitary thyroid axis.[4]. *Vidarikandhchurna* helps in reduction of symptoms like anxiety insomnia, palpitation because bio-chemical components in *Vidarikhand* include β- sitosterol, stigmasterol, duidzein, tuberosin, puerarin, Pterocarpan-tuberosin, pterocarpanone-hydroxytuberosone, two pterocarpenes-an hydrotuberosin. Apart from this, it also contains 64% carbohydrates and 10.9%proteins. Enriched with these bio active components and host of qualities like immuno- protective, rejuvenative, cardiotonic, diuretic, hypertensive, *detoxifying, digestive*, anti-inflammatory, broncho-dilatory, pain-relieving, anti-microbial properties. This medicinal plant is also used to treat heart related problems, constipation and diarrhoea.[5].

*Vidarikhand* balances *Vata* and *Pitta* in the body. *Shatavari churna* balances vitiated *vata* and *kapha*. It boosts immunity and have rejuvenating properties. *Shatavari* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactogogue, and aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic and as tonic. *Shatavrichurna* regulates mensuration cycle by balancing oestrogen and progesterone levels.[6]. Studies show that aqueous concentration of the root have indicated noteworthy changes in thyroid hormone level. Its extract decreases the levels of T3 and T4 compared the reference drug propyl thio uracil [7]. *Aswagandha* contain *Kashaya, Tikta Rasa; Laghu, Snighdha Guna; Ushna Veerya* but *Madhura Vipaka*. It possesses *Rasayana, Balya, Vrishya* properties. *Ashwagandha* has been used as an anti-inflammatory, anti-oxidative, anti-anxiety, aphrodisiac, immune-modulation, CNS depressant, hepato-protective, cardiovascular protection, adaptogenic and anti-stress [8]. All these properties may ease the syndromes of hyperthyroidism. Various studies also show that *Aswagandha* have regulating effect on thyroid glands [9]. *Brahmi Vati* is used for the treatment of anxiety insomnia, tremors in hands. It reduces both mental and physical heat (*Tapa prashamana*) and mitigating irritability and excitability. *Brahmi Vati* may offer neuroprotective effects. Studies show that the drug may protect the brain cells against oxidative stress [10].

## CONCLUSION

In *Ayurveda*, there is no disease contemporary to Subclinical hyperthyroidism but inferences can be made that it may be correlated to *Bhasmakaroga* or *Vata avarana* or imbalance in *vata, pitta and kapha doshas*. This case of subclinical hyperthyroidism was treated with holistic approach of Ayurveda based on the symptoms. The patient got symptomatic relief and also bio chemical levels of the thyroid profile were in range by *Virechana karma* and *Shamana Yoga*. But more clinical controlled studies are required to prove the effectiveness of this treatment.

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**Table No 1: Treatment Schedule**

| Serial No. | Treatment | Drugs | Dose |
|---|---|---|---|
| 1. | *Deepana-Pachana* | *PanchakolChurna*3gm BD | For 2 days |
| 2. | *Snehapana* | *Mahatiktak Ghrita* (started with initial dose of 30 ml and end dose 150 ml | For 5 days |
| 3. | *Sarvanga Abhyanga* ( external oleation) | With *Dashmoolataila* | For 3 days |
| 4. | *Sarvanga Swedana* (fomentation) | With *Dashmoolataila* | For 3 days |
| 5. | *Virechana* | *Triphalakwatha* -15gm + *Kutaki Churna*-10gm+ *Triphalakwatha* 200 ml | For 1 day |
| 6. | *PeyadiSansarjana Karma* | *Peyadi Sansarjana* | For 5 days |
| 7. | *Shamana Yoga* | 1.*Shatavari churna* 10gm+ 10 gm *Vidarigandhchurna Ksheerpaka* 2.*Ashwgandha churna*3gm BD | For 30 days |
| | | 3. *Brahmi Vati* 1 BD | For 30 days |

**Table No. 2: Thyroid Profiling**

| Thyroid Profile, Total Serum | Bio. Ref. Interval | Before Trial | After Trial |
|---|---|---|---|
| T3 | 0.70-2.04 ng/mL | 1.19 | 1.60 |
| T4 | 5.74-13.03 µU/mL | 10.38 | 11.02 |
| TSH | 0.35-5.50 µIU/mL | 0.10 | 0.4 |

Name [redacted]
Lab No. [redacted]
Ref By : Dr. sgt hospital
Collected : 25/11/2023 9:38:00AM
A/c Status : P
Collected at : AIIMS BADSA-CC
Age : 34 Years
Gender : Female
Reported : 25/11/2023 7:57:07PM
Report Status : Final
Processed at : LPL-GURGAON
CVB, Clear View Building Old DLF Colony, Sector-14 Gurgaon, Haryana-122001

Test Report

| Test Name | Results | Units | Bio. Ref. Interval |
|---|---|---|---|
| THYROD PROFILE,TOTAL SERUM (CLIA) | | | |
| T3, Total | 1.19 | ng/mL | 0.70 - 2.04 |
| T4, Total | 10.38 | µg/dL | 5.74 - 13.03 |
| TSH | 0.10 | µIU/mL | 0.35 - 5.50 |

Note
1. TSH levels are subject to circadian variation, reaching peak levels between 2 - 4 a.m. and at a minimum between 6-10 pm . The variation is of the order of 50% . hence time of the day has influence on the measured serum TSH concentrations.
2. Alteration in concentration of Thyroid hormone binding protein can profoundly affect Total T3 and/or Total T4 levels especially in pregnancy and in patients on steroid therapy.
3. Unbound fraction ( Free,T4 /Free,T3) of thyroid hormone is biologically active form and correlate more closely with clinical status of the patient than total T4/T3 concentration
4. Values <0.03 uIU/mL need to be clinically correlated due to presence of a rare TSH variant in some individuals

Interpretation

| PREGNANCY | REFERENCE RANGE FOR TSH IN µIU/mL ( As per American Thyroid Association) |
|---|---|
| 1st Trimester | 0.100 - 2.500 |
| 2nd Trimester | 0.200 - 3.000 |
| 3rd Trimester | 0.300- 3.000 |

Page 1 of 2

Name [redacted]
Lab No. : 44021317
Ref By : Dr. sgt hospital
Collected : 25/01/2024 10:58:00AM
A/c Status : P
Collected at : AIIMS BADSA-CC
Age : 34 Years
Gender : Female
Reported : 25/01/2024 8:47:07PM
Report Status : Final
Processed at : LPL-GURGAON
CVB, Clear View Building Old DLF Colony, Sector-14 Gurgaon, Haryana-122001

Test Report

| Test Name | Results | Units | Bio. Ref. Interval |
|---|---|---|---|
| THYROD PROFILE,TOTAL SERUM (CLIA) | | | |
| T3, Total | 1.60 | ng/mL | 0.70 - 2.04 |
| T4, Total | 10.02 | µg/dL | 5.74 - 13.03 |
| TSH | 0.4 | µIU/mL | 0.35 - 5.50 |

Note
1. TSH levels are subject to circadian variation, reaching peak levels between 2 - 4 a.m. and at a minimum between 6-10 pm . The variation is of the order of 50% . hence time of the day has influence on the measured serum TSH concentrations.
2. Alteration in concentration of Thyroid hormone binding protein can profoundly affect Total T3 and/or Total T4 levels especially in pregnancy and in patients on steroid therapy.
3. Unbound fraction ( Free,T4 /Free,T3) of thyroid hormone is biologically active form and correlate more closely with clinical status of the patient than total T4/T3 concentration
4. Values <0.03 uIU/mL need to be clinically correlated due to presence of a rare TSH variant in some individuals

Interpretation

| PREGNANCY | REFERENCE RANGE FOR TSH IN µIU/mL ( As per American Thyroid Association) |
|---|---|
| 1st Trimester | 0.100 - 2.500 |
| 2nd Trimester | 0.200 - 3.000 |
| 3rd Trimester | 0.300- 3.000 |

**Fig.1.Thyroid Profiling**

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**RESEARCH ARTICLE**

# On Some Properties of Summability in Arithmetic - Geometric Series

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**ABSTRACT**

The concept of summability theory has been dealt with several forms in recent years. In this paper, we present a perspective in generalizing Ramanujan summation method by considering Geometric and Arithmetic-Geometric Progressions.Geometric verifications are also included. The applications of geometric and arithmetic – geometric series on Pascal's triangle and other applications in science are also described here.

**keywords:** Geometric Series, Arithmetic – Geometric series, Ramanujan summation, Pascal's triangle

## INTRODUCTION

The concept of summability theory has been prevailing for several years as of now and has been a much studied topic. This idea has paved way for developing new branch of mathematical analysis called "Summability Theory". The purpose of this paper is to present an approach to Ramanujan summation methods for geometric and arithmetic geometric series.

**Definition**

The Ramanujan summation is defined as $R.S(\sum_{n=1}^{\infty} a_n) = \int_{-1}^{0} s_n \, dn$ (1)

where $n$ is a positive integer and $s_n$ is sum upto first $n$ terms of the divergent series $\sum_{n=1}^{\infty} a_n$ of real numbers.

**Theorem 1**

The Ramanujan summation of the geometric series $a + ar + ar^2 + \cdots + ar^{n-1} + \cdots$ is given by

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \begin{cases} \frac{a}{r-1}\left(\frac{1}{logr} - \frac{1}{rlogr} - 1\right) & if \quad r > 1 \\ \frac{a}{1-r}\left(1 - \frac{1}{logr} + \frac{1}{rlogr}\right) & if \quad 0 < r < 1 \\ -\frac{1}{2}a & if \quad r = 1 \end{cases} \tag{2}$$

where $a$ is the initial term and $r$ is the common ratio between the terms.

**Proof:**

For the geometric series , the sum to $n$ terms is given by

$$S_n = \begin{cases} \frac{a(r^n-1)}{r-1} & if\ r > 1 \\ \frac{a(1-r^n)}{1-r} & if\ 0 < r < 1 \\ na & if\ r = 1 \end{cases} \tag{3}$$

For the case$r > 1$

Substituting (3) in (1) we get,

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} \frac{a(r^n-1)}{r-1} dn = \frac{a}{r-1}\left(\frac{r^n}{logr} - n\right)_{-1}^{0} = \frac{a}{r-1}\left(\frac{1}{logr} - \frac{1}{rlogr} - 1\right)$$

For the case $0 < r < 1$

Substituting (3) in (1) we get,

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} \frac{a(1-r^n)}{1-r}\ dn = \frac{a}{1-r}\left(n - \frac{r^n}{logr}\right)_{-1}^{0} = \frac{a}{1-r}\left(1 - \frac{1}{logr} + \frac{1}{rlogr}\right)$$

For the case $r = 1$,

Substituting (3) in (1) we get,

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} (na)dn = a(\left(\frac{n^2}{2}\right)_{-1}^{0} = -\frac{1}{2}a$$

**Geometric Meaning**

This can be verified geometrically by taking $a = 1$ and scaling $r$ for few values as follows

We observe that from the shaded portion of Figure 1(a) to Figure 1(b) is that the region representing the area of $S_n$between x- axis and the interval [-1,0] lies below the x- axis and it decreases as the value of common ratio increases.

From the figure for $r = 1$, length equals to 1 and breadth equals to 1 and the area is $-\frac{1}{2}$

**Corollary 1**

The Ramanujan summation of the series $2^0 + 2^1 + 2^2 + \cdots + 2^n + \cdots$ is

$R.S(\sum_{n=1}^{\infty} a_n) = \frac{1}{2\ln 2} - 1 = -0.27865(4)$

**Proof:**

$2^0 + 2^1 + 2^2 + \cdots + 2^n + \cdots$ is a geometric series with $a = 1$ and $r = 2$

Substituting $a = 1$ and $r = 2$ in (3)

$S_n = \frac{2^n-1}{2-1} = 2^n - 1$ (5)

Substituting (5) in (1)

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} (2^n - 1)dn = \left[\frac{2^n}{\ln 2} - n\right]_{-1}^{0} = \frac{1}{2\ln 2} - 1$$

$$= \frac{1}{2(0.693147)} - 1 = -0.27865$$

**Geometric meaning**

This can be verified geometrically as follows

We observe that from the shaded portion of Figure 2 is that the region representing the area of $S_n$ between x- axis and the interval [-1,0] lies below the X- axis found to be -0.278652 which equals to $\frac{1}{2\ln 2} - 1$

**Theorem 2**

The Ramanujan summation of the arithmetic -geometric series

$$a + (a+d)r + (a+2d)r^2 + (a+3d)r^3 + \cdots + (a+(n-1)d)r^{n-1} + \cdots \quad (6)$$

is given by

$$R.S(\textstyle\sum_{n=1}^{\infty} a_n) = \begin{cases} \frac{a(1-r)+dr}{(1-r)^2} + \frac{(a-d)}{r\ln r} - \frac{d}{r(\ln r)^2} & if\ r > 0\ and\ r \neq 1 \\ -\frac{a}{2} + \frac{5d}{12} & if\ r = 1 \end{cases} \quad (7)$$

where $a$ is the initial term ,$r$ is the common ratio and $d$ is the common difference between the terms.

**Proof:**

The sum to $n$ terms of the arithmetic - geometric series is given by

$$s_n = a + (a+d)r + (a+2d)r^2 + (a+3d)r^3 + \cdots + (a+(n-1)d)r^{n-1} \quad (8)$$

$$= a(1 + r + r^2 + \cdots + r^{n-1}) + dr(1 + 2r + 3r^2 + \cdots + (n-1)r^{n-2})$$

$if\ r > 0\ and\ r \neq 1$ ,then

$$s_n = a\left(\frac{1-r^n}{1-r}\right) + dr(1 + 2r + 3r^2 + \cdots + (n-1)r^{n-2}) \quad (9)$$

Also$1 + 2r + 3r^2 + \cdots + (n-1)r^{n-2} = \frac{d}{dr}(r + r^2 + \cdots + r^{n-1}) =$

$$= \frac{d}{dr}\left(r\left(\frac{1-r^{n-1}}{1-r}\right)\right) = \frac{(n-1)r^n - nr^{n-1} + 1}{(1-r)^2} \quad (10)$$

Substituting (10) in (9) we get,

$$s_n = a\left(\frac{1-r^n}{1-r}\right) + dr\left(\frac{(n-1)r^n - nr^{n-1} + 1}{(1-r)^2}\right)$$

$$= \frac{a(1-r)+dr}{(1-r)^2} - \frac{ar^n}{1-r} + \frac{dr}{(1-r)^2}\left(r^{n-1}\left((n-1)r - n\right)\right) \quad (11)$$

Substituting (11) in (1) we get,

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} s_n dn = \int_{-1}^{0}\left(\frac{a(1-r)+dr}{(1-r)^2} - \frac{ar^n}{1-r} + \frac{dr}{(1-r)^2}\left(r^{n-1}\left((n-1)r - n\right)\right)\right) dn$$

$$= \left[\left(\frac{a(1-r)+dr}{(1-r)^2}\right)n - \frac{a}{1-r}\left(\frac{r^n}{\ln r}\right) + \frac{d(r-1)}{(1-r)^2}\left(n\frac{r^n}{\ln r} - 1\left(\frac{r^n}{(\ln r)^2}\right)\right) - \frac{dr}{(1-r)^2}\frac{r^n}{\ln r}\right]_{-1}^{0}$$

$$= \frac{a(1-r)+dr}{(1-r)^2} + \frac{a}{r\ln r} + \frac{d}{r-1}\left(\frac{1}{r\ln r} - \frac{1}{(\ln r)^2} + \frac{1}{r(logr)^2}\right) - \frac{d}{(r-1)\ln r}$$

$$= \frac{a(1-r)+dr}{(1-r)^2} + \frac{a}{r\ln r} - \frac{d}{r\ln r} - \frac{d}{r(\ln r)^2} \quad (12)$$

$If\ r = 1$, (6) becomes

$$s_n = a + (a+d) + (a+2d) + (a+3d) + \cdots + (a+(n-1)d) \quad (13)$$

Substituting (13) in (1) we get,

$R.S(\sum_{n=1}^{\infty} a_n) = \int_{-1}^{0} s_n dn = \int_{-1}^{0} \left(\frac{n}{2}(2a + (n-1)d)\right) dn$

$= \left[a\frac{n^2}{2} + \frac{d}{2}\left(\frac{n^3}{3} - \frac{n^2}{2}\right)\right]_{-1}^{0} = -\frac{a}{2} + \frac{5d}{12}$ ■

**Geometric meaning**

This can be verified geometrically by taking $a = 1$ and $d = 1$ scaling $r$ for few values as follows.
We observe that from the shaded portion of Figure 3(a) to Figure 3(d) is that the region representing the area of $S_n$ between x- axis and the interval [-1,0] lies below the x- axis.

**Corallary 1:**

If $r = e$, the Ramanujan summation of the arithmetic -geometric series becomes

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \frac{1}{e(e-1)^2}\big((a-2d) + e(d(4-e) - a)\big)$$

Also if $a = 1, d = 1$ then, $R.S(\sum_{n=1}^{\infty} a_n) = -0.0291$ approximately

Proof

Substituting $r = e$ in (7)

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \frac{a(1-e) + de}{(1-e)^2} + \frac{a-2d}{e}$$

$$= \frac{1}{e(e-1)^2}(a - 2d - de^2 - ae + 4ed)$$

$$= \frac{1}{e(e-1)^2}\big((a-2d) + e(d(4-e) - a)\big)$$

If $a = 1, d = 1$ then

$$R.S(\sum_{n=1}^{\infty} a_n) = \frac{1}{e(e-1)^2}(-e^2 + 3e - 1) = -\frac{0.23421}{8.02568} = -0.0291 \text{ approximately}$$ ■

**Applications in the Pascals' triangle**

We could see Ramanujan like summation can be applied for diagonal elements of Pascal's triangle. which is a triangular array of the binomial coefficients that has wide applications in various mathematical fields like probability theory, combinatorics and algebra.
The first eight rows of the Pascal's triangle is as follows

**Theorem 3**

The Ramanujan summation of the sum of the diagonal elements along the first slant diagonal of Pascals' triangle is $R.S(\sum_{n=1}^{\infty} a_n) = -\frac{1}{2}$ (14)

**Proof:**

The sum of the diagonal elements along the first diagonal is given by $1 + 1 + 1 + \cdots + n + \cdots$
which is a geometric progression series
Substituting $a = 1$ and $r = 1$ in (5) we get $S_n = n$ (15)
Substituting (15) in (1) we get

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} n\, dn = -\frac{1}{2}$$

■

**Geometric meaning**

This can be verified geometrically as follows

We observe that from the shaded portion of Figure 5 is that the region representing the area of $S_n$ betweenx- axis and the interval [-1,0] lies below the x- axis found to be -0.5 which equals to $-\frac{1}{2}$

**Theorem 4**

The Ramanujan summation of the sum of the diagonal elements along the second slant diagonal of Pascal's triangle is $R.S(\sum_{n=1}^{\infty} a_n) = -\frac{1}{12}$(16)

**Proof:**

The sum of the diagonal elements along the second diagonal is given by

$1 + 2 + 3 + 4 + \cdots + n + \cdots$which is an arithmetic - series where $a = 1$ and $d = 1$

The sum to n terms is given by $s_n = \frac{n}{2}(2a + (n-1)d) = \frac{n}{2}\big(2 + (n-1)\big) = \frac{n}{2}(n+1)$(17)Substituting (17) in (1) we get

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} (\frac{n}{2}(n+1)dn = -\frac{1}{12}$$

**Geometric meaning**

This can be verified geometrically as follows

We observe that from the shaded portion of Figure 6is that the region representing the area of $S_n$between x-axis and the interval [-1,0] lies below the x axis found to be −0.0833333333333.which is approximately equals to $-\frac{1}{12}$

This result is similar to Ramanujan work on $1 + 2 + 3 + \cdots = -\frac{1}{12}$

**Theorem 5**

The Ramanujan summation ofthe sum of the diagonal elements along the third slant diagonal of Pascal'triangle is $R.S(\sum_{n=1}^{\infty} a_n) = -\frac{1}{24}$ (18)

**Proof:**

The sum of the diagonal elements along the third diagonal of Pascal's triangle is given by

$1 + 3 + 6 + \cdots n + \cdots$

The sum to $n$ terms of the series is given by$S_n = \binom{n+2}{3} = \frac{n^3+3n^2+2n}{6}$ (19)

Substituting (19) in (1) we get

$$R.S\left(\sum_{n=1}^{\infty} a_n\right) = \int_{-1}^{0} (\frac{n^3 + 3n^2 + 2n}{6}\Big) dn = -\frac{1}{24} \quad \blacksquare$$

**Geometric meaning**

This can be verified geometrically as follows.

We observe that from the shaded portion of (Figure 7) is that the region representing the area of $S_n$between x- axis and the interval [-1,0] lies below the x- axis found to be −0.0416666666667 which is approximately equals to $-\frac{1}{24}$

**Other Applications in Science**

Here are some additional applications of geometric and arithmetic geometric series in various scientific fields. Scientists across various disciplines can leverage geometric and arithmetic geometric series whenever exponential growth, decay, or repeated processes with a constant factor come into play. For instances in physics, in diffraction gratings and in geometric optics, in Chemistry, Chemical Kinetics and serial dilutions, in biology, Signal Transduction, population Genetics and in other sciences, earthquake magnitudes, ecology etc

## CONCLUSION

We have discussed the Ramanujan summation for geometic and arithmetic-geometric series and we have applied this to Pascal's triangle and we got nice results. We have used Desmos graphing software tool to create graphs presented in the figures. Further we can construct Ramanujan summation for various other series also using the techniques presented in this paper.

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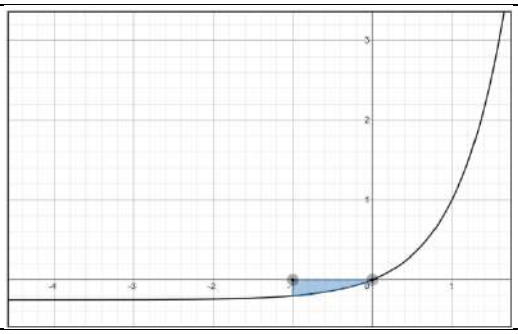

**Figure 1(a)**
**Graph for $\int_{-1}^{0} \frac{a(r^n-1)}{r-1} dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 5$**

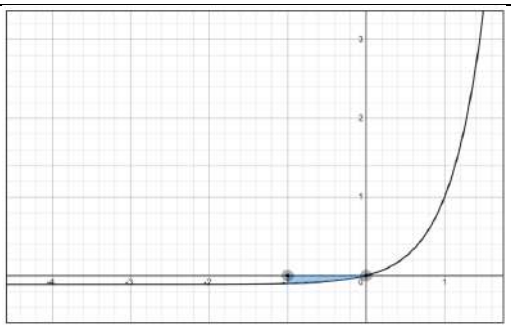

**Figure 1(b)**
**Graph for $\int_{-1}^{0} \frac{a(r^n-1)}{r-1} dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 10$**

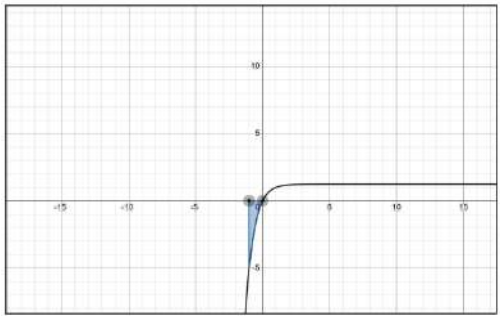

**Figure 1(c)**
**Graph for $\int_{-1}^{0} \frac{a(1-r^n)}{1-r}\ dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 0.2$**

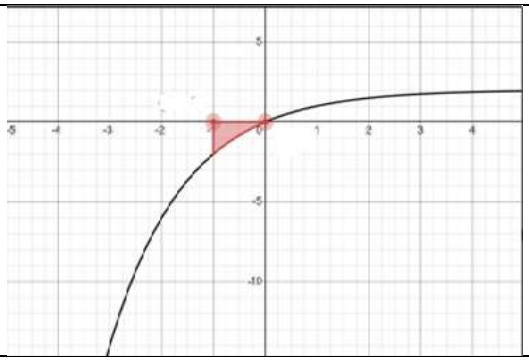

**Figure 1(d)**
**Graph for $\int_{-1}^{0} \frac{a(1-r^n)}{1-r}\ dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 0.5$**

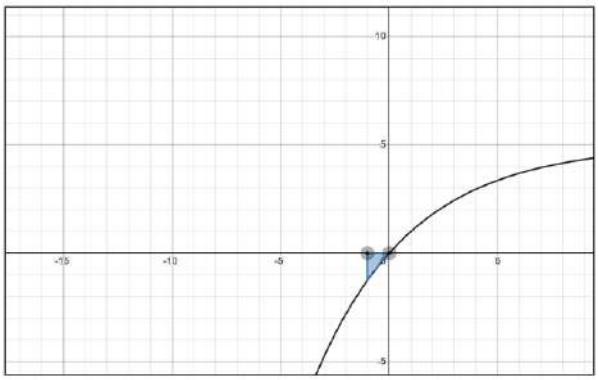

**Figure 1(e)**
**Graph for $\int_{-1}^{0} \frac{a(1-r^n)}{1-r}\ dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 0.8$**

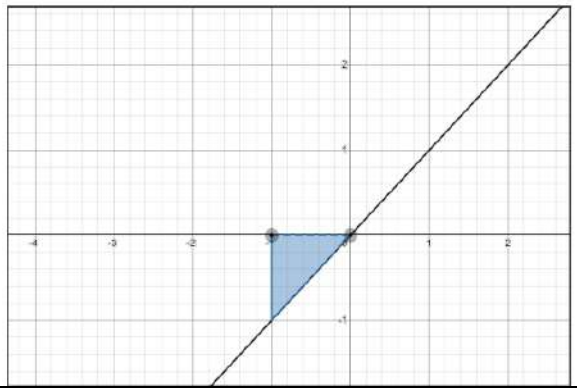

**Figure 1(f)**
**Graph for $\int_{-1}^{0} (na) dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0]for $r = 1$**

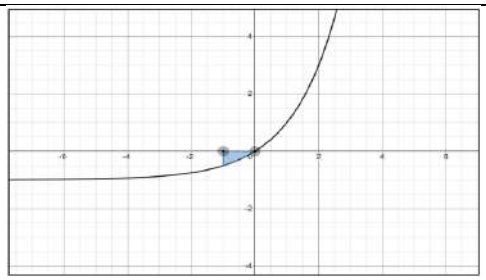

**Figure 2**
**Graph for $\int_{-1}^{0}(2^n - 1)dn$**
**showing area of $S_n$ between x- axis and the interval [-1,0]**

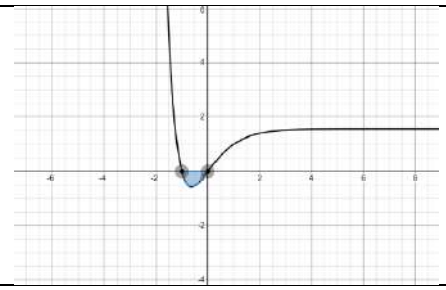

**Figure 3(a)**

**Graph for**

$$\int_{-1}^{0}\left(a\left(\frac{1-r^n}{1-r}\right) + dr\left(\frac{(n-1)r^n - nr^{n-1} + 1}{(1-r)^2}\right)\right)dn$$

**showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 0.2$**

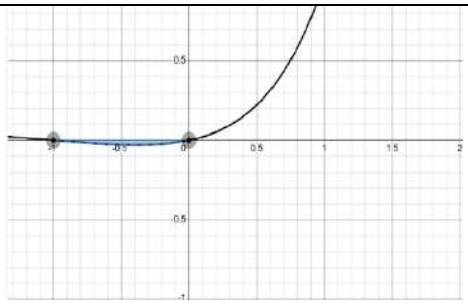

*Figure 3(b)*

*Graph for*

$$\int_{-1}^{0}\left(a\left(\frac{1-r^n}{1-r}\right) + dr\left(\frac{(n-1)r^n - nr^{n-1} + 1}{(1-r)^2}\right)\right)dn$$

*showing area of $S_n$ between x- axis and theinterval [-1,0] for $r = 0.8$*

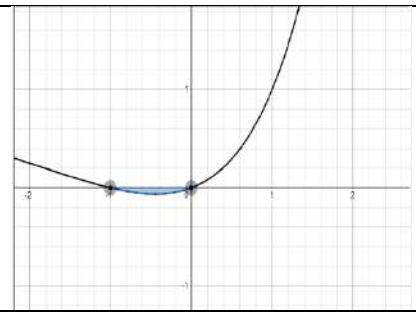

*Figure 3(c)*

*Graph for*

$\int_{-1}^{0}\left(a\left(\frac{1-r^n}{1-r}\right) + dr\left(\frac{(n-1)r^n - nr^{n-1} + 1}{(1-r)^2}\right)\right)dn$*showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 2$*

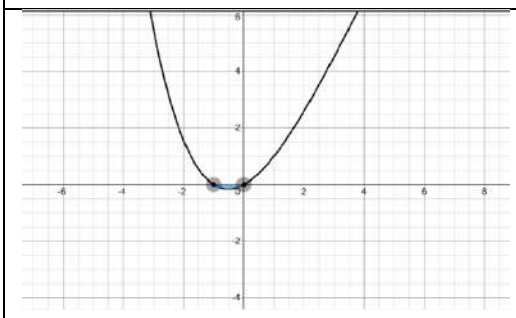

**Figure 3(d)**
**Graph for**
$\int_{-1}^{0}\left(a\left(\frac{1-r^n}{1-r}\right) + dr\left(\frac{(n-1)r^n - nr^{n-1} + 1}{(1-r)^2}\right)\right)dn$ **showing area of $S_n$ between x- axis and the interval [-1,0] for $r = 4$**

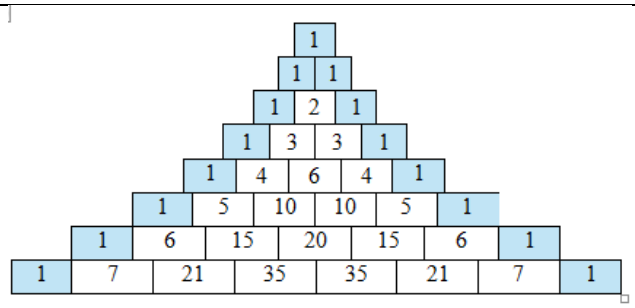


**Figure 4 Pascal's Triangle**

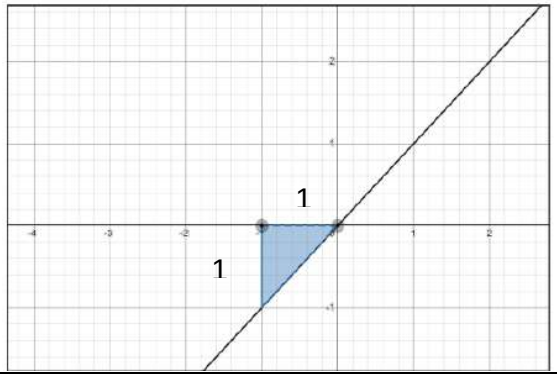


***Figure 5***
***Graph for*** $\int_{-1}^{0} \mathbf{n}\,\mathbf{dn}$
***showing area of*** $\mathbf{S_n}$ ***between x- axis and the interval [-1,0]***

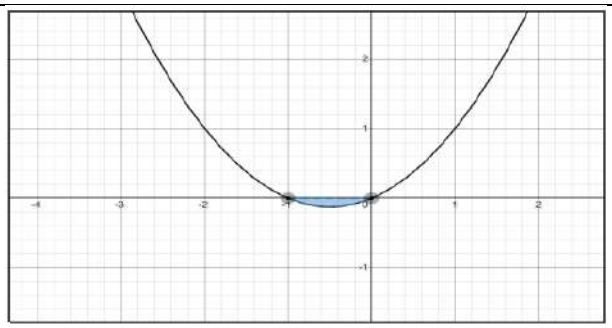

***Figure 6***
***Graph of*** $\int_{-1}^{0} (\frac{\mathbf{n}}{\mathbf{2}}(\mathbf{n}+\mathbf{1})\mathbf{dn}$
***showing area of*** $\boldsymbol{S_n}$ ***between x- axis and the interval [-1,0]***

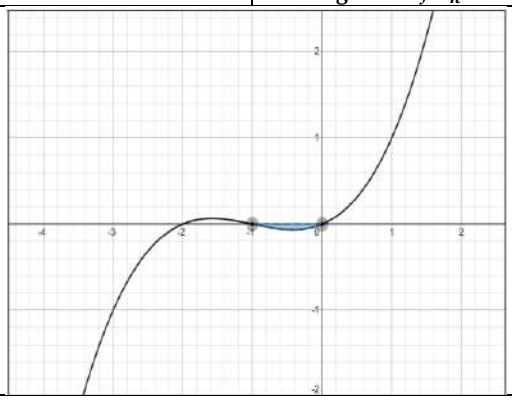

***Figure 7***
***Graph of*** $\int_{-1}^{0} (\frac{\mathbf{n^3+3n^2+2n}}{\mathbf{6}})\,\mathbf{dn}$
**showing area of** $\boldsymbol{S_n}$ **between x- axis and the interval [-1,0]**

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**RESEARCH ARTICLE**

# Optimizing Water Hyacinth Compost and Chicken Manure for Enhancing Spinach Growth: An Eco-Friendly Approach to Sustainable Agriculture

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## ABSTRACT

Sustainable agriculture practices that reduce reliance on synthetic inputs are essential for environmental conservation and food security. This study explores the potential of transforming the invasive aquatic weed, water hyacinth (*Eichhornia crassipes* (Mart.) Solms), and chicken manure, a by-product of poultry farming, into organic fertilizers to enhance spinach (*Spinacea oleracea* L.) leaf area growth. Using Design Expert Software (Version 13), the proportions of water hyacinth compost and chicken manure were optimized to maximize spinach leaf area growth. Response surface methodology and central composite design helped find the best mix (PREDICTION). This optimal point, shown by software-generated contour and 3D surface plots, was then validated by mixing the contents in tubs for spinach leaf area, showing similar results. The optimal blend of 1184.17 grams of compost and 41.72 grams of chicken manure resulted in a maximum spinach leaf area of 225 square centimeters, exhibiting the efficacy of this organic fertilizer. This approach not only promotes plant growth but also presents a sustainable waste management strategy, mitigating the ecological impact of this invasive weed. The resulting organic fertilizer blend significantly improved soil properties by reducing bulk density and increasing porosity, enhancing aeration and root penetration for water and mineral absorption. Additionally, compared to unamended soil, the higher levels of total nitrogen, phosphorus, and potassium create an ideal environment for nutrient absorption by plants.

**Keywords:** Water hyacinth compost, Chicken manure, Organic fertilizers, Sustainable agriculture, Response surface methodology

## INTRODUCTION

In the pursuit of sustainable agricultural practices, the integration of eco-friendly approaches has become increasingly crucial. One such approach involves the utilization of organic amendments derived from waste materials, such as water hyacinth compost (WHC) and chicken manure (CM). This study aims to optimize the application of these amendments through response surface methodology (RSM) to enhance spinach growth, fostering an environmentally conscious and economically viable agricultural system. The growing need for food production and the reduction of the harmful effects of synthetic fertilizers have led to the search for alternative nutrient sources [1]. Water hyacinth (*Eichhornia crassipes* (Mart.) Solms ), an invasive aquatic plant, is a valuable resource for nutrient-rich compost due to its high biomass production and nutrient uptake capacity [2]. Controlled composting processes can convert this weed into organic fertilizer, aiding sustainable waste management and nutrient recycling. CM, a by-product of poultry farming, is known for its high content of nitrogen, phosphorus, and potassium [3]. These alternatives present promising solutions for sustainable agriculture, addressing both environmental concerns and the need for effective fertilization. Numerous studies have explored the use of WHC and CM, either individually or in combination, for enhancing the growth and yield of various crops, such as maize [4], tomatoes, brinjal, and chilli [5,6], and cowpea [7]. Converting this weed into nutrient-rich compost through controlled processes offers environmental sustainability, soil health improvement, nutrient recycling, enhanced crop yield and quality, and integrated waste management strategies.

Efforts have been made to optimize synthetic fertilizer with organic fertilizer combinations to maximize plant growth and minimize environmental impacts. For example, Moradi *et al*., 2024 [8] turned NPK (synthetic), cow manure, and vermicompost into a nutrient-rich compost blend for growing quinoa (*Chenopodium quinoa* Willd.). Similarly, RSM was used to optimize the application rate of nitrogen and phosphorus (synthetic fertilizers) and cattle manure in wheat production [9]. While previous studies optimized combinations of inorganic and organic fertilizers using RSM with three or more components, this study uniquely focuses on using only two variables: water hyacinth compost and chicken manure as sole organic fertilizers, excluding synthetic inputs. By converting water hyacinth into nutrient-rich compost and combining it with chicken manure, this research demonstrates that organic fertilizers alone can effectively support the growth of spinach. This approach, using RSM combined with a Central Composite Design (CCD) to statistically optimize the proportions of these two factors for optimal spinach growth, aims to address concerns about the sufficiency of organic fertilizers. It promotes sustainable agriculture systems, environmental conservation, resource efficiency, and food security. The findings offer valuable insights for farmers, researchers, and policymakers interested in adopting sustainable and eco-friendly agricultural practices.

## MATERIALS AND METHODS

**Seed material and Germination Procedure**

Spinach (*Spinacia oleracea*) seeds obtained from a local market were stored at 30% relative humidity and 13°C until use. This study took place at J.K. College in Purulia, West Bengal, under ex-vitro environmental conditions. The seeds were subjected to pre-treatment by soaking in water for 24 hours to enhance germination. Germination conditions were maintained at a temperature of 21°C, with a light cycle of 12 hours of light and 12 hours of darkness, and a humidity level of 50%. Seeds were placed on the moist filter paper in the petri dishes and covered with lids to maintain humidity. These dishes were then placed inside a germination cabinet to provide a controlled environment. Daily monitoring was conducted to observe germination progress, and the number of germinated seeds was recorded.

**Preparation of WHC**

The study on compost optimization was conducted using contained within galvanized wire cages mesh size (3/4 inch) of 3 feet diameter 4 feet height, secured with twine or quick-release knots placed in an open area and set on an

elevated platform. To prepare compost, a controlled composting method was employed, relying solely on organic material derived from water hyacinth and naturally occurring microbes from soil not previously enriched with organic amendments. Water Hyacinth was collected from "Rishi Nibaran Sayer", a lake in Purulia district, West Bengal. The composting process lasted 3 months (25 June-5 October 2023).The compost site was established with a base layer of fractured brick, gravel, stone, and sand for proper drainage and aeration. Shredded water hyacinth served as the primary composting material, spread evenly over the base layer. The composting pile was contained within galvanized wire cages, secured with twine or quick-release knots. Moisture levels and consecutive turning (5 days interval) were maintained using a fertilizer hose-end sprayer, and a tarp covered the pile to retain heat and moisture. Temperature monitoring was conducted with a compost temperature probe, with readings taken twice daily to ensure the core temperature remained between 45°C and 57°C. The pile was turned if the temperature exceeded this range, with cooler outer layers moved towards the center for even decomposition. pH and moisture readings were taken once daily, with adjustments made as necessary. Once the compost achieved a stable temperature range, dark crumbly texture, and earthy smell, it entered the curing phase, typically lasting 1-4 weeks. Before application to soil, the compost was screened to remove any large, un-decomposed pieces.

**CM Preparation**

CM was collected from a local poultry farm and air-dried on trays for four days to reduce moisture. The partially dried manure was then sterilized in an autoclave at 121°C and 15 psi for 15 minutes to remove pathogens. After sterilization, it was sun-dried for another three days to become odorless and evaporate any remaining moisture. The dried chicken manure was sieved through a 2.00-mm mesh to achieve the desired particle size and homogeneity. The final product was ready for blending with compost in varying proportions to create an organic fertilizer mixture aimed at enhancing spinach growth.

**Soil Quality Analysis**

Soil physical parameter- bulk density and porosity [10], chemical parameter such as pH [11], TN [12], Available P [13], Available K [14], were analyzed to assess soil quality.

**Analysis of Variance (ANOVA) study for Quadratic Model** (via main effects, interactions, or quadratic effects with relative importance of each term for model's accuracy and reliability**):** The ANOVA study [15] for the quadratic model evaluates the significance and adequacy of the fitted model in representing the relationship between the response variable (leaf area) and the independent variables (water hyacinth compost and chicken manure). The ANOVA assesses the null hypothesis (H0) that all model terms are insignificant ($\beta = 0$) against the alternative hypothesis (H1) that at least one term is significant ($\beta \neq 0$). The F-test determines if the variation caused by each term is significantly greater than the residual error, with p-values indicating the significance level. The ANOVA table lists the sources of variation (linear, quadratic, interaction terms), degrees of freedom (df), sum of squares, mean square (MS), F-value (ratio of MS for each term to residual MS), and p-value. The F-value and p-value help identify significant model terms influencing the response variable and assess model adequacy through lack-of-fit tests. The analysis guides the selection of relevant terms for constructing an optimized quadratic model, supporting the interpretation and validation of the RSM results.

**Experimental Design**

In this experiment, Spinach seeds were surface-sterilized before planting in 10-inch pots prepared with the soil mixtures. The seeds were sown at a depth of 0.5 inches, with proper spacing and watering for germination and early development. RSM was employed using a CCD [16] to optimize the parameters for spinach growth, with Design-Design Expert v.13 software used for statistical analysis. The independent variables investigated were WHC and CM, with three levels each (600, 1200, and 1800 gm for compost; 12, 24, and 48 gm for CM) (Table-3). A full factorial combination resulted in 9 treatment combinations, plus two additional treatments (pure soil and pure compost), for a total of 11 treatments. Each treatment was replicated three times, leading to 33 experimental units. A completely randomized design was used, with treatments randomly assigned to the experimental units. The response variable

(spinach growth) was measured in terms of leaf area. Appropriate statistical methods, such as ANOVA, were used to evaluate the significance of the main effects and their interaction on the response variable.

**Analysis of Plant Morphological Parameters**

Spinach plants were harvested after 50 days of sowing, and growth measures (leaf area) were used to assess. We use IBM SPSS Statistics 25 for statistical analysis. To determine the least significant difference between treatment means, ANOVA was used with a significance level of $p < 0.05$.

**Statistical Optimization of Spinach growth:**

Our experimental data were fitted into this second-order quadratic equation, including individual and cross-interaction effects among all variables:

$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 + \beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{32} + \beta_{44}X_{42}$, where, Y is the predicted response; $\beta_0$ is the constant term; $\beta_1$, $\beta_2$, $\beta_3$, and $\beta_4$ are the regression coefficients for linear effects; $\beta_{12}$, $\beta_{13}$, $\beta_{14}$, $\beta_{23}$, $\beta_{24}$, and $\beta_{34}$ denote the regression coefficients for interaction effects; and $\beta_{11}$, $\beta_{22}$, $\beta_{33}$, and $\beta_{44}$ are the regression coefficients for quadratic effects [17]. The response value Y was the leaf area of the spinach plant in relation to the two variables. Analysis through software, the regression of the experimental data and generate response surface graphs. Correlation coefficients "R" and determination coefficients $R^2$ were calculated to evaluate the performance of the regression equation. The fitted polynomial equation was visualized through 3D surface plots. Significance of the model was determined by ANOVA. The interactive relationship between significant variables was explored through response surface and contour plots. RSM was applied for regression and graphical analysis of the obtained data.

## RESULTS

**Soil characterization**

30 soil samples used for this experiment and bulk density, porosity, pH, nutrient content (TN, P, K) were characterized. The results are shown in the Table-1. These parameters provide baseline information about the physical and chemical properties of soil, indicating compaction level, aeration status, and initial nutrient levels, which can be compared after applying organic amendments to evaluate their effectiveness in enhancing soil fertility and interpreting subsequent results.

**Composting Process**

The composting process went through distinct phases with varying pH, temperature, and moisture content. The initial mesophilic stage lasted three weeks with pH 7.0-7.9, moderate temperature around 37°C, and moisture declining from 92% to 79.8%. The subsequent thermophilic phase peaked at 49°C with pH stabilized at 7.9 and moisture dropped to 68%. During the 8-15 week cooling and curing phases, temperature decreased to 25°C, pH dropped to 7.2, and moisture reached 30%. The optimal compost blend of WHC and CM for maximizing spinach leaf area was identified and analyzed for nutrient content and bulk density.

**Soil Properties Comparison**

The optimized WHC and CM blend significantly improved soil properties that are mentioned in Table-2.

**Optimizing Spinach Growth through Response Surface Methodology: A Statistical Approach: Response surface methodology and optimization**

In this study, RSM combined with CCD, a two-level factorial design with 3 center points, were used to evaluate the effects of two independent variables on the dependent variable, mentioned in Table-3. A quadratic model explored the mathematical relationship between variables. The design incorporated five coded levels with 11 experimental runs to analyze the interaction effects of individual, quadratic, and cross-product terms of the variables in a

randomized order. This statistical approach aimed to identify the optimal combination of WHC and CM for maximizing spinach leaf area. The CCD matrix and the observed leaf area responses for spinach plant growth are shown in Table 4. The ANOVA results for the quadratic model applied to the experimental data offer insights into the model's significance and the impact of individual factors on spinach leaf area, as shown in Table 5.

**Fit Statistics**

The C.V assesses the reliability of the statistical model by comparing the estimated standard error to the mean value of the observed response. Table 6 provides an overview of the ANOVA summary of fit statistics. A C.V. value below 10% is preferred. The Predicted $R^2$ of 0.7963 aligns closely with the Adjusted $R^2$ of 0.9100, with a difference of less than 0.2. Adequate Precision evaluates the signal-to-noise ratio, where a ratio above 4 is ideal. The ratio of 14.912 signifies a strong signal, indicating the model's suitability for exploring the design space. The C.V. value is less than 10% (4.36%), indicating that the model is also highly significant.

**Final Equation in Terms of Coded Factors:**

The final equation for the leaf area of the spinach plant, expressed in terms of coded factors, is provided below:

Sqrt (Leaf Area) = 15.29 + 0.4181 * A + 0.3884 * B - 0.1398 * AB - 0.2482 * $A^2$ - 1.28 * $B^2$,

Where A denotes WHC and B represents CM (in grams). The coded factors equation is useful for predicting responses based on specific levels of each factor. Typically, the higher levels of the factors are coded as +1, and the lower levels are coded as -1. This coded equation predicts responses based on factor levels and helps determine their relative influence through coefficient comparison. The constant term (15.29) represents the predicted square root of leaf area when all independent variables (A, B, AB, $A^2$, $B^2$) are zero. The coefficient of A (0.4181) indicates that for each unit increase in the water hyacinth compost application rate, with other variables constant, the square root of leaf area increases by 0.4181 units. Similarly, the coefficient of B (0.3884) suggests that each unit increase in the CM application rate increases the square root of leaf area by 0.3884 units. The coefficient of AB (-0.1398) shows the interaction effect between WHC and CM, with the negative value indicating their combined effect is less than their individual effects' sum. The coefficient of $A^2$ (-0.2482) indicates that increasing water hyacinth compost beyond a certain point may decrease the square root of leaf area, suggesting a non-linear relationship. Likewise, the coefficient of $B^2$ (-1.28) implies that higher CM application rates may reduce the square root of leaf area, also suggesting a non-linear relationship.

**Final equation in terms of actual factors:**

The final equation in terms of actual factors for the square root of spinach leaf area is:

Sqrt (Leaf Area) = 8.79038 + 0.002740 * Compost + 0.274069 * Chicken Manure - 0.000013 * Compost * Chicken Manure - 6.89444E-07 * $Compost^2$ - 0.003949 * $Chicken\ Manure^2$.

This equation can predict responses based on specific levels of WHC and CM in their original units. However, the coefficients here are adjusted for the units and cannot be directly compared to assess relative importance. Notably, the larger negative quadratic coefficient for chicken manure (-0.003949) compared to compost (-6.89444E-07) suggests a more pronounced non-linear relationship between chicken manure application rate and the square root of leaf area. This implies that excessive CM application may decrease leaf area more severely than excessive compost application, highlighting the importance of optimizing both amendments proportions.

**Residual plots**

These plots help identify differences between experimentally derived and predicted values. Fig. 1 illustrates the relationship between predicted and actual leaf area of spinach plants after 50 days of harvesting. The plot indicates that the quadratic model is significant for analyzing response and input variables. The data points closely follow the linear trend line, suggesting the model accurately estimates leaf area response. The model exhibits a good fit, evidenced by the evenly distributed data points along the line with minimal deviations.

**Response surface plot analysis:**

Contour plots optimize multiple variables and identify significant parameters, visually mapping the experimental area for maximum response. They also reveal important interactions between factors that could lead to the desired outcome. The quadratic response surface model is examined using 3D response surfaces and 2D contour plots, where one variable is held constant while the other two are varied within experimental ranges. Figures 2 and 3 present contour and 3D surface plots visualizing the interaction effects of WHC and CM on spinach leaf area response. The plots show higher leaf area values concentrated in the upper-right region, suggesting higher levels of both factors are favorable. The smooth contours indicate a well-behaved response surface within the experimental ranges. Analysis predicts the optimum point for maximum leaf area (approximately 225 $cm^2$) at 1184.17 gm WHC and 41.72 gm CM, marked by the red point on the contour plot. The innermost and outermost contours represent leaf areas of around 200 $cm^2$ and 180 $cm^2$, respectively. The distinct peak in the response surface signifies a quadratic relationship, with an optimal factor combination maximizing leaf area, highlighting the importance of strategic application rate adjustments for enhancing spinach growth.

**Point Prediction**

In Table-7, the 95% confidence interval for the mean leaf area is between 201.483 $cm^2$ and 249.851 $cm^2$, indicating that there is 95% confidence that the true mean falls within this range. The 95% tolerance interval for 99% of the population spans from 125.641 $cm^2$ to 352.286 $cm^2$, meaning that 99% of individual observations or future leaf area values are expected to fall within these limits with 95% confidence. To ensure accurate results, new experiments were conducted using the optimal input variable combination of 1184.17 gm WHC and 41.72 gm CM, as determined by the optimization process.

**Compare predicted and experimental values**

The predictive model's performance was evaluated by calculating the percentage error or residual between the predicted (225 $cm^2$) and experimental leaf area values (10 sets: 221.6, 221.44, 226.2, 224.4, 224.4, 223.6, 223.2, 227.2, 221.5, and 222 $cm^2$) at optimal input variable levels. To validate the robustness of RSM model through experimentation, we can employ statistical test like one-sample t-test or confidence interval analysis were employed using the new experimental data. The one-sample t-test determines if the mean experimental leaf area significantly differs from the predicted 225 $cm^2$, with the H0 stating no difference and the H1suggesting a difference. The assumptions include independent and randomly sampled data, and approximate normality. Alternatively, a confidence interval for the mean difference between predicted and experimental values can assess the statistical significance.

## DISCUSSION

The results of this study demonstrate the potential of utilizing organic waste materials, such as water hyacinth compost and chicken manure, as eco-friendly fertilizers for enhancing the growth of spinach plants. The amended soil exhibited significantly improved physical and chemical properties compared to the control soil. The bulk density decreased from 1.194 g/cc to 0.825 g/cc, while porosity increased from 45.28% to 68.87%, indicating a lighter, more porous structure for increased water infiltration, root growth, and is desirable for overall plant growth and health [18]. Additionally, nutrient levels substantially increased: nitrogen from 357.8 mg/kg to 1120 mg/kg (tripled), phosphorus from 23.60 mg/kg to 106 mg/kg, and potassium from 110 mg/kg to 143 mg/kg. These enhancements in soil quality, structure, and fertility by incorporating compost suggest potential benefits for better plant growth and agricultural productivity [19]**.** Begum *et al*., 2022 reviewed potential of water hyacinth, noting a substantial upsurge on its effect on soil and plant properties [20].

The composting process involved distinct phases with varying conditions: an initial mesophilic stage with pH 7.0-7.9, temperature around 37°C, and moisture decreasing from 92% to 79.8% over three weeks; a thermophilic phase peaking at 49°C with pH 7.9 and moisture dropping to 68%; and cooling and curing phases over 8 to 15 weeks where temperature fell to 25°C, pH to 7.2, and moisture to 30%. The influence of temperature and moisture content regimes

on the aerobic microbial activity in composting blends was observed by Liang *et al*., 2003 [21]. Bulk density decreased from previous, while porosity increased, enhancing aeration and water infiltration. Nutrient levels remarkably increased. The organic matter and nutrient-rich compost reduced soil compaction, improved structure, and increased essential nutrient availability for plant growth [22].

The application of RSM and CCD enabled the systematic optimization of the WHC and CM levels within specified ranges, providing insights into the optimal proportions required for maximizing spinach leaf area. Table-3 defines the low and high levels of these variables, while Table-4 displays the CCD matrix and observed leaf area responses. Experimental runs followed the CCD matrix, with observed leaf area responses varying from 75.36 $cm^2$ to 241.22 $cm^2$ across different variable combinations. These findings offer insights into how changes in compost and chicken manure levels influence. The statistical analysis revealed that the quadratic model effectively captured the relationship between the two independent variables and the dependent variable. RSM and CCD were also utilized to optimize various agricultural and biological processes. The yield and quality of French bean (*Phaseolus vulgaris* L) were enhanced by Kumar *et al*., 2020 [23] through the optimization of the effects of Plant Growth-Promoting Rhizobacteria and silicon fertilization. Similarly, a two-variable CCD and RSM were used by Prakash and Srivastava, 2005 [24] to study the optimization of carbon (sucrose/glucose) and nitrogen ($NO_3^-/NH_4^+$ ratio) in *Azadirachta indica* suspension culture. Optimal conditions were identified in both studies, with the quadratic model accurately representing the relationship between the independent and response variables, enabling the optimization of French bean yield, quality, and media.

In table-5 shows the impact of WHC and CM application rates on leaf area. The model overall was statistically significant ($p = 0.0022$), indicating that at least one factor or interaction significantly influenced leaf area. However, the main effects of WHC (A-Compost, $p = 0.1382$) and CM (B-Chicken manure, $p = 0.1287$), as well as their interaction (AB, $p = 0.6104$), were not statistically significant at the 5% level. The quadratic effect of WHC ($A^2$, $p = 0.3775$) was also not significant, whereas the quadratic effect of CM ($B^2$, $p = 0.0040$) was, suggesting a non-linear relationship with leaf area. Therefore, while the model and the quadratic effect of CM are significant, the individual contributions of WHC and CM, along with their interaction, are not statistically significant**.** In Table-6, the high coefficient of determination ($R^2 = 0.9550$) and low coefficient of variation (C.V. = 4.36%) suggest the model's good fit and reliability. ANOVA results confirm the significance of the quadratic model (p-value = 0.0022), pointing to that it is capable of accurately predicting the response within the experimental range. Some research papers revealed similar conclusion such as high $R^2$ values observed in each study—0.974 for maximizing auxin production by *Pantoea agglomerans* C1 [25], 0.9915 for optimization of phenolic compound extraction from pineapple peel waste [26] and 0.9772 for production of the xylanase enzyme by a fungal strain [27]—indicate that the quadratic models could account for a significant portion of the variability in the responses. Likewise, the low C.V., ranging from 2.74% to 4.50%, demonstrates the accuracy and consistency of the experimental findings. The quadratic models relevency was further confirmed by ANOVA, with all trials yielding p-values of less than 0.0001. This statistical confirmation proved the robustness of the models and the dependability of the findings. The quadratic model developed in this study represented by the equation –Sqrt (Leaf Area) = $15.29 + 0.4181 * A + 0.3884 * B - 0.1398 * AB - 0.2482 * A^2 - 1.28 * B^2$; is a reliable method for predicting spinach leaf area. The constant term (15.29) predicts the square root of leaf area, while A (0.4181) and B (0.3884) shows positive impacts of WHC and CM, while the coefficient of AB (-0.1398) reflects the interaction between the two. Excessive amounts of either WHC or CM reduce leaf area as indicated by coefficients $A^2$ (-0.2482) and $B^2$ (-1.28).

The predicted vs. actual plot (Fig. 1) demonstrates a strong positive correlation between the predicted and observed leaf area values, supporting the validity and reliability of the model. Although a few data points depart slightly from the trend line, the overall plot demonstrates the ability of model to effectively represent the underlying relationships. The predicted mean and median leaf area values are closely aligned, suggesting a relatively symmetric distribution of the response variable. The small confidence interval for the mean leaf area (about 201.483 $cm^2$) shows acceptable precision in predicting the real mean, while the broader tolerance interval (roughly 249.851 $cm^2$) (See Table-7) incorporates heterogeneity in individual data. These measures give useful insights into the central tendency,

uncertainty, and variability of the leaf area response based on the fitted model, allowing for inferences regarding predicted values, uncertainties, and probable outliers. Overall, the quadratic model proposed in this work provides a dependable and practical method for estimating spinach leaf area using an organic fertilizer combination of water hyacinth compost and chicken manure. The model's prediction capacity, as well as the statistical metrics presented, helps us comprehend sustainable agricultural techniques that enhance plant development while also conserving the environment.

The contour plots and 3D surface plots from the RSM analysis visually depict the interaction effects of the independent variables on the response. These plots aid in identifying the optimal factor combination to maximize spinach leaf area. Various studies have employed similar plots from RSM to visualize the interaction effects between independent variables and the response variable and also proven valuable in identifying optimal combinations of factors for achieving the desired response. RSM is employed to determine the optimal combination of factors for maximizing a particular outcome, which include extraction of phenolic compounds from pomegranate peel [28], production of enzymes by a bacterial strain [29], *Scenedesmus* sp. biomass production in meat processing wastewater by nutrient uptake [30], and cassava yield [31]. The ANOVA results show that CM has a substantial effect on spinach leaf area, with a high F-value of 25.42 for its quadratic term. Regarding the t-value, the anticipated t-test statistic is -2.2621, with degrees of freedom of 9. The results indicated no statistically significant difference between the experimental mean leaf area (223.554 $cm^2$) and the projected value (225 $cm^2$) with a p-value of 0.05. In accordance with Confidence Interval Analysis, the mean difference between experimental and predicted outcomes has a 95% confidence interval (0.004, -2.896) (See Table-8). Because this interval contains zero, there is no significant difference between the averages at the 0.05 significance level. The t-test and confidence interval analysis demonstrate that the anticipated and experimental results are consistent that supports the dependability of the RSM model and optimization outcomes from Design Expert 13.

## CONCLUSIONS

The study demonstrated that using water hyacinth compost and chicken manure significantly enhanced soil properties and spinach growth. The composting process optimized through Response Surface Methodology and Central Composite Design revealed distinct phases with varying pH, temperature, and moisture content, resulting in an ideal compost blend. The optimized compost application reduced soil bulk density and increased porosity and nutrient levels. Statistical analysis confirmed the quadratic model's reliability, indicating that optimal compost and manure levels significantly improved spinach leaf area. Residual and response surface plots validated the model's accuracy, showing high correlation between predicted and actual values. The integration of these eco-friendly organic amendments aligns with sustainable agriculture principles, reducing dependence on synthetic fertilizers and promoting efficient resource use. This approach is particularly beneficial for small-scale farmers, enhancing crop yield and contributing to environmental conservation. This study underscores the potential of organic waste materials in improving soil health and agricultural productivity.

**Conflicts of interest:** The authors declare no conflict of interest.

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**Illustrations:**

**Table-1: Characterization on Bulk Density, Porosity, and Nutrient Content (TN, P, K) in Control soil, values which are shown mean value ±SE.**

| | Bulk density(g/cc) | Porosity (%) | pH | TN (mg/kg) | P(mg/kg) | K(mg/kg) |
|---|---|---|---|---|---|---|
| Control Soil | 1.194±0.13 | 45.28±0.49 | 7.2 | 357.8±0.63 | 23.60±0.11 | 110±0.78 |

**Table-2: Impact of Compost Addition on Bulk Density, Porosity, and Nutrient Content (TN, P, K) in amended soil, values which are shown mean value ±SE.**

| | Bulk density(g/cc) | Porosity (%) | pH | TN (mg/kg) | P(mg/kg) | K(mg/kg) |
|---|---|---|---|---|---|---|
| Amended soil | 0.825±0.53 | 68.87±0.19 | 7.2 | 1120±0.69 | 106±0.33 | 143±0.7 |

**Table 3: Levels of Independent and Dependent Variables in CCD for maximizing the leaf area expansion of spinach plants**

| Name | Units | Type | Low | High |
|---|---|---|---|---|
| Water Hyacinth Compost (Independent variable ) | gm | Factor | 600 | 1800 |
| Chicken manure (Independent variable ) | gm | Factor | 12 | 48 |
| Leaf Area (Dependent Variables or response ) | $cm^2$ | Response | 75.36 | 241.22 |

**Table 4: CCD Matrix and Observed Leaf Area Responses for Spinach Plant Growth:**

| Std | Run | Factor 1<br>A: Compost<br>gm | Factor 2<br>B: Chicken Manure<br>gm | Response -Leaf area<br>$cm^2$ |
|---|---|---|---|---|
| 4 | 1 | 0 | 0 | 75.36 |
| 6 | 2 | 600 | 12 | 185 |
| 9 | 3 | 600 | 24 | 189 |
| 3 | 4 | 600 | 48 | 195 |
| 11 | 5 | 1200 | 12 | 197 |
| 5 | 6 | 1200 | 24 | 208 |
| 1 | 7 | 1200 | 48 | 212 |
| 10 | 8 | 1800 | 12 | 192 |
| 8 | 9 | 1800 | 24 | 241.22 |
| 2 | 10 | 1800 | 48 | 204.75 |
| 7 | 11 | 1200 | 0 | 116 |

**Table 5: presents the ANOVA results for the quadratic model of spinach growth (in terms of leaf area).**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 36.32 | 5 | 7.26 | 21.23 | 0.0022 | significant |
| A-Compost | 1.06 | 1 | 1.06 | 3.11 | 0.1382 | |
| B-Chicken manure | 1.13 | 1 | 1.13 | 3.31 | 0.1287 | |
| AB | 0.1009 | 1 | 0.1009 | 0.2949 | 0.6104 | |
| $A^2$ | 0.3206 | 1 | 0.3206 | 0.9370 | 0.3775 | |
| $B^2$ | 8.70 | 1 | 8.70 | 25.42 | 0.0040 | |
| Residual | 1.71 | 5 | 0.3421 | | | |
| Cor Total | 38.04 | 10 | | | | |

**Table 6: Summary of ANOVA Fit Statistics**

| | | | |
|---|---|---|---|
| Std. Dev. | 0.5849 | $R^2$ | 0.9550 |
| Mean | 13.41 | Adjusted $R^2$ | 0.9100 |
| | | Predicted $R^2$ | 0.7963 |
| C.V. % | 4.36 | Adeq Precision | 14.9118 |

**Table-7: Summary Statistics for Spinach Leaf Area Measurements Including Confidence and Tolerance Intervals**
Two-sided Confidence = 95% Population = 99%

| Solution 1 of 100 Response | Predicted Mean | Predicted Median* | Observed | Std Dev | SE Mean | 95% CI low for Mean | 95% CI high for Mean | 95% TI low for 99% Pop | 95% TI high for 99% Pop |
|---|---|---|---|---|---|---|---|---|---|
| Leaf Area | 225.016 | 224.674 | 223.554 | 17.5415 | N/A | 201.483 | 249.851 | 125.641 | 352.286 |

**Table 8: Results of One-Sample t-test for Validation**

| Test | Result |
|---|---|
| One-Sample t-test | The calculated t-value falls in the outside range of -2.262 to 2.262, failing to reject the null hypothesis (H0). |
| Assumptions | •The experimental leaf area values are independent and randomly sampled.<br>•The leaf area values are approximately normally distributed. |
| Sample mean ($\bar{x}$) | 223.554 cm^2 |
| Sample standard deviation (s) | 2.021397756 cm^2 |
| Test statistic (t) | -2.2621 |
| Degrees of freedom | 9 |
| Critical t-value | ±2.262 |
| Confidence Interval | (0.004, -2.896) |
| Conclusion | The difference between the mean of the experimental leaf area values and the predicted value is statistically significant at the 0.05 level. |

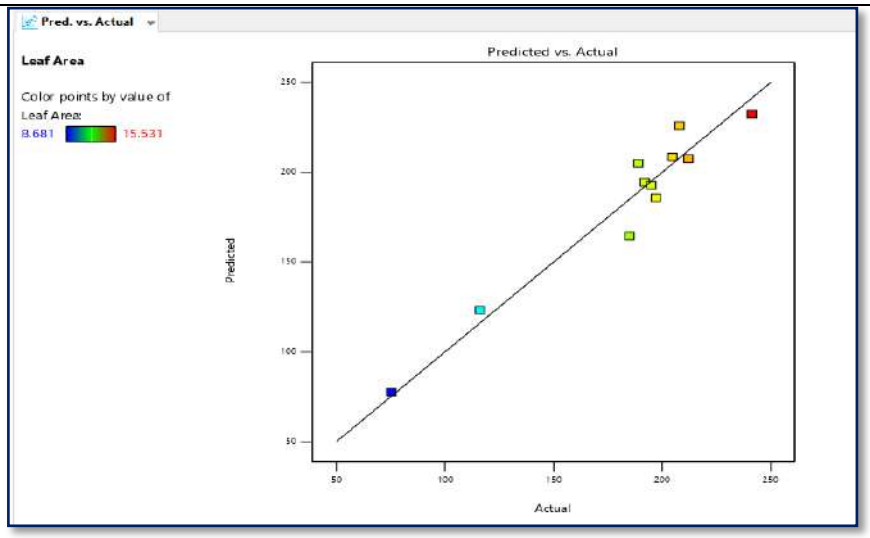


**Fig. 1. Predicted vs actual plot of residuals.**

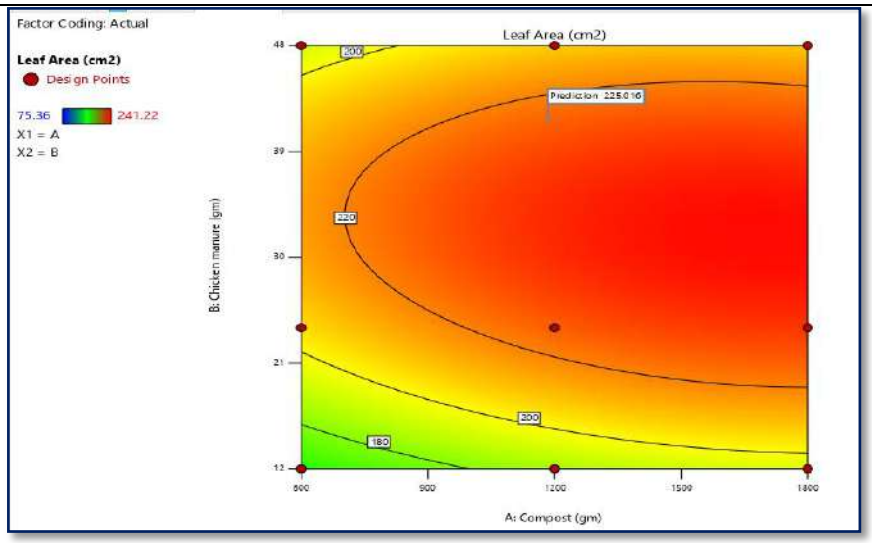


**Fig. 2. Graphical representation for response surface optimization by plotting leaf area (cm²) contour plot for (A) Compost (Water Hyacinth Compost) (gm) and (B) Chicken manure (gm).**

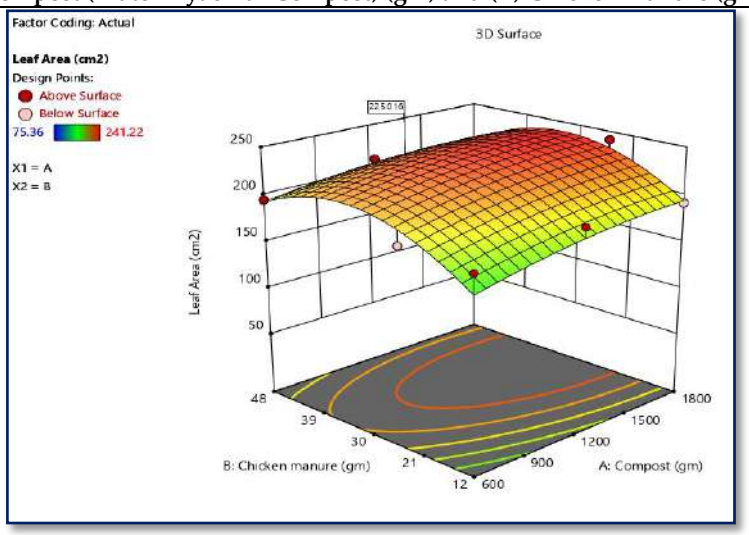


**Fig. 3. Graphical representation for response surface optimization by plotting leaf area (cm²) against a 3D plot for (A) Compost (Water Hyacinth Compost) (gm) and (B) Chicken manure (gm).**

**[**The curvature of the 3D response surface suggests the presence of non-linear relationships and potential interactions between the two factors and the response variable (Leaf Area). The curved shape indicates that the effects of the factors on the response are not purely additive, and there may be synergistic or antagonistic interactions between the factors. The optimum region or factor combination that corresponds to the highest levels of the response variable is located in the upper-right corner of the surface. This region represents high levels of both A: Compost and B: Chicken manure, suggesting that increasing the levels of these two factors may lead to increased Leaf Area.]

**Abbreviations**: RSM, Response Surface Methodology; CCD, Central Composite Design; WHC, water hyacinth compost; CM, chicken manure; ANOVA, Analysis of Variance; df, degrees of freedom; MS, mean square; CV, coefficient of variation; H0, Null hypothesis; Alternative hypothesis, H1; C.V., coefficients of variation; TN, Total Nitrogen, P, Phosphorus; K, Potassium.

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**RESEARCH ARTICLE**

# An Improved CRITIC-MULTIMOORA Approach for Kidney Transplant under Pythagorean Neutrosophic

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**ABSTRACT**

This article revolves around specific research that depicts about CRITIC-MULTIMOORA approach in a Pythagorean neutrosophic environment. A novel score function and distance measure are proposed for a Pythagorean neutrosophic, which is a fundamental concept, utilized in fuzzy Logic systems and few theories are applied to determine the similarity or membership between the elements. Using the Criteria Importance Through the Inter-Criteria Correlation (CRITIC), the Weightage of the objectives is determined. In addition to that, the multi-objective optimization on the basis of ratio analysis, following the full multiplicative form (MULTIMOORA) method, applied to create a stand-by list for the kidney recipients. At the threshold, a numerical model is provided to evaluate the feasibility of the method proposed.

**Keywords:** Pythagorean neutrosophic fuzzy set, score function, distance measure, multi objective decision making.

## INTRODUCTION

Xenograft or transplantation is one of the most crucial surgical procedures operated between a donor and recipient. Kidney was the first organ transplanted in 1954. In contemporary scenario more than 1 lakh 57 thousand organs transplanted; out of which kidney is the most transplanted organ; 80% of the adult population is aware of organ

donation. As a result, a list and itinerary of donors and recipients provided an undeniable procedure to meet out the future surgical requirements. Yager introduced the idea of the Pythagorean Fuzzy Set (PFS) [15]. The prerequisite is that the total squared of membership and non-membership must be less than or equal to one. Smarandache developed the concept of the degree of dependence between components of the fuzzy set and the neutrosophic set first. [7]. The Pythagorean Neutrosophic Set (PNS), had become familiar in recent years. It is a new concept with dependent components, that can be either member or non-member with the requirement, in which the square sum of the two components would not exceed 2, where the square sum of the degrees of membership and non-membership is Pythagorean and does not exceed 1. The degree of indeterminacy is independent. The concept of Multi-Objective Optimization based on Ratio Analysis (MOORA) was first introduced by Brausers and Zavadskas. It is the process of maximizing a conflicting goal or goals, while adhering to certain limitations. The MOORA method is applicably easy and computation time is meager. By including full multiplicative form, Brausers and Zavadskas ameliorated the MOORA method to MULTIMOORA. The three sections of MULTIMOORA are the Full Multiplicative Form System (FMFS), Reference Point System (RPS), and Ratio System (RS). Using the MULTIMOORA method, EsraAytac Adali and Aysegaultus Isik talked about a laptop selection problem [6].

A new distance measure and score function for Pythagorean fuzzy sets were proposed by Chao Huang in his Pythagorean fuzzy MULTIMOORA method, which is an extension of the original MULTIMOORA method [3]. Additionally, a practical example of producing a solid-state disk is provided to illustrate the process. The Pythagorean Neutrosophic number's score function was defined by M. Palanikumar and K. Arulmozhi [9]. The Pythagorean neutrosophic number's distance measure was provided by Jansi Rajan and Mohana krishnaswamy [8]. Diakoulaki *et al*. proposed the CRITIC method and It's a technique for figuring out the objective weight. Shakeel zafar *et al*. talked about the blockchain evaluation process using the CRITIC weight method [14]. Discussing the CRITIC-EDAS method in a fermata fuzzy environment is done by Arunodaya Raj Mishra *et al* [1]. Dragan Pamucar *et al* [5]. presented a paper that used the CRITIC method and fuzzy rough numbers. Muhammad Akram talked about the CRITIC-EDAS method in a Pythagorean setting [10].Nasrin *et al* [11] present a novel approach to weight kidney allocation criteria based on multi-expert fuzzy reasoning. As mentioned by Dalia M. Atallah *et al*., kidney transplant outcomes can be predicted using matching to determine which patient is a good fit for the donor [4]. Bernadette Li *et al* [2] investigate the survival of recipients after a deceased donor kidney transplant. The article focuses on giving a CRITIC-MULTIMOORA method to create a waiting list for recipients based on the Pythagorean Neutrosophic set. The paper is structured as follows: The basic definitions like Pythagorean neutrosophic set (PN), Distance measure and score function are covered in section 2. Methodology for the CRITIC-MULTIMOORA method is given in section 3. A real-life example of creating a waiting list for organ recipients is given in section 4. The results and discussion of this study are presented in section 5. The final section of the paper concludes with the conclusion.

**Preliminaries**

**Definition 1**

Let $X$ be a universe of discourse. A Pythagorean neutrosophic set (PN) $N$ on $X$ isdefined as$N = \{\langle x, \Phi_N(x), \Psi_N(x), \text{Ĵ}_N(x)\rangle | x \in X\}$, where $0 \le \Phi_N^2(x) + \Psi_N^2(x) + \text{Ĵ}_N^2(x) \le 2$, and the limits of each elements belongs to [0,1]. $\Phi_N(x), \Psi_N(x), \text{Ĵ}_N(x)$ denotes degree of membership, non-membership and indeterminacy.

**Definition 2**

Let$A = \{\langle x_i, \Phi_A(x_i), \Psi_A(x_i), \text{Ĵ}_A(x_i)\rangle | x \in X\}$ and $B = \{\langle x_i, \Phi_B(x_i), \Psi_B(x_i), \text{Ĵ}_B(x_i)\rangle | x \in X\}$ be two PN sets. Let $w_i (i = 1, 2, \dots n)$ be weightage of an element $x_i (i = 1, 2, \dots n)$. Where $w_i \ge 0$ and $\sum_{i=1}^{n} w_i = 1$. Then the weighted distance measure is given as

$$d_\gamma(A, B) = \left\{\frac{1}{3}\sum_{i=1}^{n} w_i \left[\left|\Phi_A^2(x_i) - \Phi_B^2(x_i)\right|^\gamma + \left|\Psi_A^2(x_i) - \Psi_B^2(x_i)\right|^\gamma + \left|\text{Ĵ}_A^2(x_i) - \text{Ĵ}_B^2(x_i)\right|^\gamma\right]\right\}^{\frac{1}{\gamma}}$$

Where, $\gamma > 0$.When $\gamma = 1$ gives the Hamming distance

$$d(A, B) = \left\{\frac{1}{3}\sum_{i=1}^{n} w_i \left[\left|\Phi_A^2(x_i) - \Phi_B^2(x_i)\right| + \left|\Psi_A^2(x_i) - \Psi_B^2(x_i)\right| + \left|\text{Ĵ}_A^2(x_i) - \text{Ĵ}_B^2(x_i)\right|\right]\right\}$$

For convenience $\Phi_A(x_i), \Psi_A(x_i), \text{Ĵ}_A(x_i)$ can be represented as $\Phi_A, \Psi_A, \text{Ĵ}_A$.

**Definition 3**

For any PN set $A = \{\Phi_A, \Psi_A, Ɲ_A\}$ the score and accuracy function are defined as

$S_A = \Phi_A^2 - \Psi_A^2 - Ɲ_A^2$, $\&E_A = \Phi_A^2 + \Psi_A^2 + Ɲ_A^2$

Where, $-1 \le S_A \le 1$, & $0 \le E_A \le 2$.

**Improved Score & Accuracy Function**

**Definition 4**

For any PN set $A = \{\Phi_A, \Psi_A, Ɲ_A\}$ the proposed score (S(A)) and accuracy (E(A)) function are defined as

$$S(A) = \frac{2 + \Phi_A^2 - \Psi_A^2 - Ɲ_A^2}{4}, \& \ E(A) = \frac{2 + \Phi_A^2 + \Psi_A^2 + Ɲ_A^2}{4}$$

Where, $0 \le S(A) \le 1$, $\&0 \le E(A) \le 1$

**Proposed Distance Measure**

**Definition 5**

Let $A =< \Phi_A(x_i), \Psi_A(x_i), Ɲ_A(x_i) >$ and $B =< \Phi_B(x_i), \Psi_B(x_i), Ɲ_B(x_i) >$ any two PN set. Then the proposed PN weighted distance measure defined as

$$d(A,B) = \frac{1}{4n}\sum_{i=1}^{n} w_i \begin{bmatrix} \left|\Phi_A^2(x_i) - \Phi_B^2(x_i)\right| + \left|\Psi_A^2(x_i) - \Psi_B^2(x_i)\right| + \left|Ɲ_A^2(x_i) - Ɲ_B^2(x_i)\right| \\ +max\{\left|\Phi_A^2(x_i) - \Phi_B^2(x_i)\right|, \left|\Psi_A^2(x_i) - \Psi_B^2(x_i)\right|, \left|Ɲ_A^2(x_i) - Ɲ_B^2(x_i)\right|\} \end{bmatrix}$$

Where $w_i \ge 0$ and $\sum_{i=1}^{n} w_i = 1$.

**PNF-CRITIC-Multimoora Approach**

This section revolves around, The CRITIC-MULTIMOORA technique is developed using a novel Pythagorean neutrosophic fuzzy distance measure and a score function. The following multi-objective problem is resolved using this method. Assume the multi-objective problem has n alternatives $\{\alpha_1, \alpha_2, \ldots\ldots \alpha_n\}$ and m objectives$\{\beta_1, \beta_2, \ldots\ldots \beta_n\}$. The objectives can be dived into two types (i.e.) objectives to be maximized and objectives to be minimized. The weight for each objective is denoted by $w = (w_1, w_2, \ldots\ldots w_m)$, $\sum_{j=1}^{m} w_j = 1$. The Pythagorean neutrosophic fuzzy matrix is given below

$$Z = (Z_{ij})_{n\times m} = \begin{bmatrix} Z_{11} & Z_{12} & \cdots & Z_{1m} \\ Z_{21} & Z_{22} & \cdots & Z_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ Z_{n1} & Z_{n2} & \ldots & Z_{nm} \end{bmatrix}$$

Here $Z_{ij}$ is a PNFN.

**A. PNF-CRITIC method**

Using the PNF-CRITIC method first, assess each objective's weight

Step 1: Calculate the score value for every element in Z using the score function to create the score matrix.

$$S = (S(Z_{ij}))_{n\times m} = \begin{bmatrix} S(Z_{11}) & S(Z_{12}) & \cdots & S(Z_{1m}) \\ S(Z_{21}) & S(Z_{22}) & \cdots & S(Z_{2m}) \\ \vdots & \vdots & \ddots & \vdots \\ S(Z_{n1}) & S(Z_{n2}) & \ldots & S(Z_{nm}) \end{bmatrix} (1)$$

Step 2: Normalize the score matrix with best and worst values as follows

$$\widetilde{S(Z_{ij})} = \begin{cases} \dfrac{S(Z_{ij}) - \min\limits_i S(Z_{ij})}{\max\limits_i S(Z_{ij}) - \min\limits_i S(Z_{ij})}, & j \in O.Max \\ \dfrac{\max\limits_i S(Z_{ij}) - S(Z_{ij})}{\max\limits_i S(Z_{ij}) - \min\limits_i S(Z_{ij})}, & j \in O.Min \end{cases} \quad (2)$$

*O. Max - Objective to be maximum

*O. Min - Objective to be minimum

Where, max $S(Z_{ij})$ and min $S(Z_{ij})$ represents the best and worst values respectively.

Step 3: Compute the Standard Deviation for every objective using the formula below.

$$\sigma_j = \sqrt{\frac{\sum_{i=1}^{n}\left(\widetilde{S(Z_{ij})} - \overline{E_j}\right)^2}{n}} \quad (3)$$

Where, $\overline{E_j} = \frac{\sum_{i=1}^{n} \widetilde{S(Z_{ij})}}{n}$.

Step 4: Assessing the comparison matrix between objectives by using the correlation coefficient

$$r_{jt} = \frac{\sum_{i=1}^{n}\left(\widetilde{S(Z_{ij})} - \overline{E_j}\right)\left(\widetilde{S(Z_{ij})} - \overline{E_t}\right)}{\sqrt{\sum_{i=1}^{n}\left(\widetilde{S(Z_{ij})} - \overline{E_j}\right)^2 \left(\widetilde{S(Z_{ij})} - \overline{E_t}\right)^2}}, \qquad (j, t = 1, \ldots, m) \quad (4)$$

Step 5: Obtain the information value of each objective

$C_j = \sigma_j * I_v$ (5)

Where, $I_v = \sum_{t=1}^{m}\left(1 - r_{jt}\right)$.

Step 6: The following step determines each objective's weight.

$$w_j = \frac{C_j}{\sum_{j=1}^{m} C_j} \quad (6)$$

**PNF-MULTIMOORA Approach**

In general, PNF-MULTIMOORA has three sections, (i.e.) PNF-RS, PNF-RPS, PNF-FMFS. Here this approach is applied to create the ranking list in the following steps:

Step1: The PNF-RS uses $S(Z_{ij})$ to determine the ranking of alternatives

$$H_1(\alpha_i) = \sum_{j=1}^{k} w_j \, S(Z_{ij}) - \sum_{j=k+1}^{m} w_j \, S(Z_{ij}) \quad (7)$$

- Here, $w_j$ is Weight of objective $\beta_j$.
- $K$ - denotes Number of objectives to be maximized.
- $m - k$ - denotes number of objectives to be minimized.
- $H_1(\alpha_i)$ - denotes ranking values of alternatives $\alpha_i$.

Alternatives can be arranged as per the ranking values (i.e.) in descending order of ranking values.

$N_1 = \{N_1(\alpha_1), N_1(\alpha_2), .., N_1(\alpha_n)\}$

Step 2: Based on$(S(Z_{ij}))_{n\times m}$ and PNF distance measure, the ranking values of alternatives for PNF-RP is computed as follows,

$$H_2(\alpha_i) = \max_j w_j \frac{d\left(Z_j^+, Z_{ij}\right)}{d\left(Z_j^+, Z_j^-\right)} \quad (8)$$

- Here, $Z_j^+, Z_j^-$ - denotes the greatest and lowest values of objectives with respect to$\beta_j$.
- $d\left(Z_j^+, Z_{ij}\right)$ - denotes the PNF distance measure between$Z_j^+, Z_{ij}$.
- $d(Z_j^+, Z_j^-)$ - denotes the PNF distance measure between $Z_j^+, Z_j^-$.

Greatest $Z_j^+$and Lowest $Z_j^-$values of each objective are defined as

$$Z_j^+ = \begin{cases} \max_j\{Z_{ij}\}, & if\ objective\ \beta_j\ need\ to\ be\ maximized \\ \min_j\{Z_{ij}\}, & if\ objective\ \beta_j\ need\ to\ be\ minimized \end{cases}$$

&

$$Z_j^- = \begin{cases} \min_j\{Z_{ij}\}, & if\ objective\ \beta_j\ need\ to\ be\ maximized \\ \max_j\{Z_{ij}\}, & if\ objective\ \beta_j\ need\ to\ be\ minimized \end{cases}$$

Here alternatives are arranged in ascending order since smaller valued alternative is better.

$N_2 = \{N_2(\alpha_1), N_2(\alpha_2), \ldots\ldots N_2(\alpha_n)\}$

Step 3: Based on$(S(Z_{ij}))_{n\times m}$, the PNF-FMFS is computed as follows:

$$H_3(\alpha_i) = \frac{\sqrt[k]{\prod_{j=1}^{k}\left(2-\left(1-S(Z_{ij})\right)^{w_j}\right)}}{\sqrt[m-k]{\prod_{j=k+1}^{m}\left(2-\left(1-S(Z_{ij})\right)^{w_j}\right)}} \quad (9)$$

Alternates are arranged in descending order as higher valued alternates are the better one.

$N_3 = \{N_3(\alpha_1), N_3(\alpha_2), \ldots\ldots N_3(\alpha_n)\}$

Step4: Since this method gets results from three systems, it should undergo aggregation. In this step, for each objective $\beta_j, (j = 1,2,3,..,n)$ there will be a ranking value $H_l(\alpha_i), (l = 1,2,3)$ and ranking order $N_l(\alpha_i)$.After aggregation the problem end up with two matrices which is ranking value matrix (RVM) $H = (H_l(\alpha_i))_{n\times 3}$and ranking order matrix (ROM) $N = (N_l(\alpha_i))_{n\times 3}$.

$$H = (H_l(\alpha_i))_{n\times 3} = \begin{bmatrix} H_1(\alpha_1) & H_2(\alpha_1) & H_3(\alpha_1) \\ H_1(\alpha_2) & H_2(\alpha_2) & H_3(\alpha_2) \\ \vdots & \vdots & \vdots \\ H_1(\alpha_n) & H_2(\alpha_n) & H_3(\alpha_n) \end{bmatrix}$$

$$N = (N_l(\alpha_i))_{n\times 3} = \begin{bmatrix} N_1(\alpha_1) & N_2(\alpha_1) & N_3(\alpha_1) \\ N_1(\alpha_2) & N_2(\alpha_2) & N_3(\alpha_2) \\ \vdots & \vdots & \vdots \\ N_1(\alpha_n) & N_2(\alpha_n) & N_3(\alpha_n) \end{bmatrix}$$

Normalization of RVM is done as follows,

$$H^- = \left(H_l^-(\alpha_i)\right)_{n\times 3} = \begin{bmatrix} H_1^-(\alpha_1) & H_2^-(\alpha_1) & H_3^-(\alpha_1) \\ H_1^-(\alpha_2) & H_2^-(\alpha_2) & H_3^-(\alpha_2) \\ \vdots & \vdots & \vdots \\ H_1^-(\alpha_n) & H_2^-(\alpha_n) & H_3^-(\alpha_n) \end{bmatrix}$$

Here

$$\left(H_l^-(\alpha_i)\right) = \frac{H_l(\alpha_i)}{\sqrt{\sum_{i=1}^{n}(H_l(\alpha_i))^{\,2}}} \quad (10)$$

When determining the ranking outcomes of alternatives, the normal MULTIMOORA method will consider ranking orders of alternatives only. There are no reasonable results to be obtained by considering only ranking orders [16]. Using the Borda rule from [3] to create the ranking order by combine the rank values.

$$R(\alpha_i) = \left(H_1^-(\alpha_i) * \frac{n - N_1(\alpha_i) + 1}{\frac{n(n+1)}{2}}\right) - \left(H_2^-(\alpha_i) * \frac{N_2(\alpha_i)}{\frac{n(n+1)}{2}}\right) + \left(H_3^-(\alpha_i) * \frac{n - N_3(\alpha_i) + 1}{\frac{n(n+1)}{2}}\right) (11)$$

The alternatives can be arranged in descending order by their $R(\alpha_i)$ value.

**Case Study**

Patients suffering in final-stage of renal disease can be subjected to kidney transplantation for better life. These patients may have multiple complications due to the kidney complications like cardiovascular disease, gastrointestinal disease, and so on. Hence, patients are prescribed to undergo several diagnoses that includes the capacity of patient's surgical tolerance and immune suppression that follows transplant surgery. Doctors use Glomerular Filtration Rate (GFR) to measure the kidney function. GFR value below 60 for three months or more than that indicates the person have kidney disease and less than or equal to 15 means the person is suffering with end stage kidney failure. They are suggested for dialysis or transplant to live. The criteria for transplantation are follows: ABO matching is a test for blood group matching because, the blood group of recipients should match with donor. The blood group O is considered as universal donor and AB is universal recipients, who could receive organ or blood from any blood group. HLA stands for Human Leukocyte Antigen; there are 100 different antigens present in our body. Out of 100 it is shown that 6 antigens matching is the most important for transplantation. In many cases a kidney from an elderly donor will not suit for young recipients. Age difference between donor and recipients is also a pivotal factor for post-surgical survival. There are two types of transplantations 1) Living donor transplantation, 2) Cadaveric transplantation. In living donor transplantation, the donor can be family members, friends. Here the donor should be in proper physical and mental health. Cadaveric transplantation will happen when the donor is declared as brain dead. In this case, after getting a proper approval from close relatives the organs will be removed for transplantation. To get the organ one should register in the organ transplant waiting list. United Network for Organ

Sharing (UNOS) will match the donated kidney with recipients in waiting list. It might be less or longer than this, the typical waiting period for a kidney transplant is two to three years.
In this section, a case about preparation of waiting list for kidney recipients illustrated on the basis of PNF-CRITIC-MULTIMOORA method.

Let us assume there are five recipients denoted $R = \{R1, R2, R3, R4, R5\}$.While ordering the recipients one should consider the following objectives: ABO compatibility($\beta_1$), HLA match ($\beta_2$), Enrolment period ($\beta_3$), Age difference ($\beta_4$), Kidney working percentage ($\beta_5$). Here $\beta_1, \beta_2, \beta_3$are objectives to be maximized. $\beta_4, \beta_5$ are objectives to be minimized. Next, each objective's weight is determined by PNF-CRITIC technique using Excel. The PNF decision matrix was created using the linguistic terms (Table. I) and is provided in Table II.

Step 1: To create the score matrix (Table. III), determine the score value for each element in Table. II.

$$S(Z_{ij}) = \frac{2 + \Phi^2_{(Z_{ij})} - \Psi^2_{(Z_{ij})} - J^2_{(Z_{ij})}}{4}$$

Step 2: Using the best and worst values Normalize Table. III.

| | | | | | |
|---|---|---|---|---|---|
| Best | 0.685 | 0.5675 | 0.5125 | 0.625 | 0.5125 |
| Worst | 0.275 | 0.275 | 0.3175 | 0.3625 | 0.3175 |

Step 3: Utilising the formula < =STDEV (Column j)>, determine the Standard Deviation for every column.

| | | | | | |
|---|---|---|---|---|---|
| SD | 0.379825 | 0.401045 | 0.452148 | 0.459389 | 0.412095 |

Step 4: Use the correlation coefficient to compare each objective. (t=1, j, m, etc.). For every element in Table IV, apply <CORREL (Column j, Column t) >.
Step 5: Apply equation (5) to obtain the information value Cj.

| | | | | | |
|---|---|---|---|---|---|
| Cj | 1.6220 | 2.0068 | 2.2300 | 2.0547 | 2.1606 |

Step 6: Finally, the weightage of each objective is calculated using equation (6).

| | | | | | |
|---|---|---|---|---|---|
| $W_j$ | 0.1610 | 0.1992 | 0.2214 | 0.2039 | 0.2145 |

Now applying the PNF-MULTIMOORA approach
Step 1: The PNF-RS uses above score matrix and the equation (7) to produce the ranking value as $H_1(\alpha_1) = 0.0227$, $H_1(\alpha_2) = 0.0802$, $H_1(\alpha_3) = 0.0906$, $H_1(\alpha_4) = 0.0884$, $H_1(\alpha_5) = 0.0553$
The ranking order is given by $N_1 = \{\alpha_3, \alpha_4, \alpha_2, \alpha_5, \alpha_1\}$

Step 2: The PNF-RPS uses above score matrix and the equation (8) to produce the ranking value as
$H_2(\alpha_1) = 0.2040$, $H_2(\alpha_2) = 0.2145$, $H_2(\alpha_3) = 0.1992$, $H_2(\alpha_4) = 0.2214$, $H_2(\alpha_5) = 0.2214$

The ranking order is given by $N_2 = \{\alpha_3, \alpha_1, \alpha_2, \alpha_4, \alpha_5\}$.

Step 3: The PNF-FMFS uses above score matrix and the equation (9) to produce the ranking value as
$H_3(\alpha_1) = 0.9634$, $H_3(\alpha_2) = 0.9963$, $H_3(\alpha_3) = 1.0099$, $H_3(\alpha_4) = 0.9986$, $H_3(\alpha_5) = 0.9869$

The ranking order is given by $N_3 = \{\alpha_3, \alpha_4, \alpha_2, \alpha_5, \alpha_1\}$
Step 4: combining all the ranking values and ranking orders results in

$$H = (H_l(\alpha_i))_{5\times3} = \begin{bmatrix} 0.0227 & 0.2040 & 0.9634 \\ 0.0802 & 0.2145 & 0.9963 \\ 0.0906 & 0.1992 & 1.0099 \\ 0.0884 & 0.2214 & 0.9986 \\ 0.0553 & 0.2214 & 0.9869 \end{bmatrix} \& N = (N_l(\alpha_i))_{5\times3} = \begin{bmatrix} 5 & 2 & 5 \\ 3 & 3 & 3 \\ 1 & 1 & 1 \\ 2 & 4 & 2 \\ 4 & 5 & 4 \end{bmatrix}$$

Normalization transforms the matrix as

$$H^{-} = (H_{\bar{l}}^{-}{}_{l}(\alpha_i))_{5\times 3} = \begin{bmatrix} 0.1410 & 0.4297 & 0.4347 \\ 0.4969 & 0.4519 & 0.4495 \\ 0.5616 & 0.4197 & 0.4557 \\ 0.5479 & 0.4664 & 0.4506 \\ 0.3428 & 0.4664 & 0.4453 \end{bmatrix}$$

The final ranking score is computed by the equation (11) as follows,
$R(\alpha_1) = -0.0189$, $R(\alpha_2) = \ \ 0.0989$, $R(\alpha_3) = \ \ 0.3111$, $R(\alpha_4) = \ \ 0.1419$, $R(\alpha_5) = -0.0504$

The final ranking is $\alpha_3 > \alpha_4 > \alpha_2 > \alpha_1 > \alpha_5$.

## RESULTS AND DISCUSSION

This section elaborates the brief interpretation of the results. According to the results, recipient 3 is clearly the most preferred recipient. Similarly, recipients 2, 4, 5, & 1 will be in second, third, fourth and fifth place, respectively. The utility of a chronologically organized waiting list can reduce the risk of deniable deceases, symptoms of heavy sickness, and mismatches between the donor and the recipient. Whenever we find some donors the waiting list is recalculated through all the recipients. If not, doctors suggest the recipient to do dialysis until they get the right donor. Every Hospital and doctors will have different set of opinions, but this type of ranking is the best choice for finding a good match between recipients and donors.

## CONCLUSION

This research-based case study is an approach to CRITIC-MULTIMOORA, improved through a novel score function and distance measure under the Pythagorean Neutrosophic Fuzzy environment. The waiting list for kidney recipients is used as an example to demonstrate the method's efficiency. In the future, the proposed method will be developed to solve diurnal problems almost in many sciences and medicine sector.

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**Table. 1**

| **Similarity (%)** | **Convert Linguistic terms into PNN** | | |
|---|---|---|---|
| | ***Recommend*** | ***Abbreviation*** | ***PNN*** |
| 0.1 – 10 | Not Conceivable | NC | <0.05, 0.65, 0.80> |
| 10.1 – 20 | Very Slightest Conceivable | VSC | <0.15, 0.60, 0.75> |
| 20.1 – 30 | Slightest Conceivable | SC | <0.25, 0.55, 0.70> |
| 30.1 – 40 | Below Average Conceivable | BAC | <0.35, 0.50, 0.65> |
| 40.1 – 50 | Equal Conceivable | EC | <0.45, 0.45, 0.60> |
| 50.1 – 60 | Average Conceivable | AC | <0.55, 0.40, 0.55> |
| 60.1 – 70 | Above Average Conceivable | AAC | <0.65, 0.35, 0.50> |
| 70.1 – 80 | Highly Conceivable | HC | <0.75, 0.30, 0.45> |
| 80.1 – 90 | Very Highly Conceivable | VHC | <0.85, 0.25, 0.40> |
| 90.1–99.9 | Flawless Conceivable | FC | <0.95, 0.20, 0.35> |

**Table 2 Pair wise Comparison among Recipient to Objectives**

| Recipients | $\boldsymbol{\beta}_1$ | $\boldsymbol{\beta}_2$ | $\boldsymbol{\beta}_3$ | $\boldsymbol{\beta}_4$ | $\boldsymbol{\beta}_5$ |
|---|---|---|---|---|---|
| $\alpha_1$ | VSC | BAC | AC | VHC | SC |
| $\alpha_2$ | HC | AAC | BAC | EC | AAC |
| $\alpha_3$ | AC | VSC | AAC | BAC | BAC |
| $\alpha_4$ | FC | HC | SC | VHC | BAC |
| $\alpha_5$ | EC | AC | SC | AAC | SC |

**Table 3 Score Matrix**

| Recipients | $\boldsymbol{\beta}_1$ | $\boldsymbol{\beta}_2$ | $\boldsymbol{\beta}_3$ | $\boldsymbol{\beta}_4$ | $\boldsymbol{\beta}_5$ |
|---|---|---|---|---|---|
| $\alpha_1$ | 0.275 | 0.3625 | 0.46 | 0.625 | 0.3175 |
| $\alpha_2$ | 0.5675 | 0.5125 | 0.3625 | 0.41 | 0.5125 |
| $\alpha_3$ | 0.46 | 0.275 | 0.5125 | 0.3625 | 0.3625 |

| | | | | | |
|---|---|---|---|---|---|
| $\alpha_4$ | 0.685 | 0.5675 | 0.3175 | 0.625 | 0.3625 |
| $\alpha_5$ | 0.41 | 0.46 | 0.3175 | 0.5125 | 0.3175 |

**Table 4 Normalized Matrix**

| Recipients | $\boldsymbol{\beta}_1$ | $\boldsymbol{\beta}_2$ | $\boldsymbol{\beta}_3$ | $\boldsymbol{\beta}_4$ | $\boldsymbol{\beta}_5$ |
|---|---|---|---|---|---|
| $\alpha_1$ | 0.0000 | 0.2991 | 0.7308 | 0.0000 | 1.0000 |
| $\alpha_2$ | 0.7134 | 0.8120 | 0.2308 | 0.8190 | 0.0000 |
| $\alpha_3$ | 0.4512 | 0.0000 | 1.0000 | 1.0000 | 0.7692 |
| $\alpha_4$ | 1.0000 | 1.0000 | 0.0000 | 0.0000 | 0.7692 |
| $\alpha_5$ | 0.3293 | 0.6325 | 0.0000 | 0.4286 | 1.0000 |

**Table. 5 Pair wise Comparison among Objectives**

| | $\boldsymbol{\beta}_1$ | $\boldsymbol{\beta}_2$ | $\boldsymbol{\beta}_3$ | $\boldsymbol{\beta}_4$ | $\boldsymbol{\beta}_5$ |
|---|---|---|---|---|---|
| $\boldsymbol{\beta}_1$ | 1 | 0.6878 | -0.5277 | 0.0796 | -0.5100 |
| $\boldsymbol{\beta}_2$ | 0.6878 | 1 | -0.9242 | -0.4033 | -0.3642 |
| $\boldsymbol{\beta}_3$ | -0.5277 | -0.9242 | 1 | 0.3698 | 0.15005 |
| $\boldsymbol{\beta}_4$ | 0.0796 | -0.4033 | 0.3698 | 1 | -0.5187 |
| $\boldsymbol{\beta}_5$ | -0.5100 | -0.3642 | 0.1500 | -0.5187 | 1 |

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**RESEARCH ARTICLE**

# Petrography and Geochemistry of NNW-SSE Trending Dolerite Dykes around Piriyapatna Taluk, Mysore District, Karnataka

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**ABSTRACT**

The differential stress in crustal rocks often results in deep fractures. Crustal dynamics is the source of energy which is expressed as a wide variety of deformation and displacement of brittle rocks. Characterization of such stress domains in brittle rocks is possible to a large extent by geometrical analysis of the orientation and mode of occurrence of hypabyssal rocks, exemplified by dykes and sills. Archean Dharwar crat on has abundant distribution of mafic dyke swarms, but all previous works mainly focus on EDC dykes. Dyke swarms of WDC yet needs detailed investigation and geochemical studies. Here we present preliminary study on the petrography and geochemistry such WDC Dyke bodies exposed in and around the Piriyapatna taluk of Mysore district in order to reveal petrogenetic implications based on the mineralogical and textural characters.

**Keywords:** Dolerite dykes, Meta-dolerites, Piriyapatna, Petrography, Whole-rock geochemistry.

## INTRODUCTION

Mafic dykes are considered as the indicators of crustal evolution, tectonic process, and continental drift history. Numerous studies on Achaean dykes have been carried out from the late 90s till date. Those studies paved the way for understanding the petrographic, geochemical, and geochronological characteristics of mafic dykes of peninsular India (Devaraju T.C 1995, Radhakrishna et al. 2007).Detailed studies on Neo-proterozoic dykes from Dharwar craton

exemplified the significance of mafic dykes of peninsular India in the evolution of lithospheric mantle (Ikramuddin & Steuber 1976). Numerous geoscientists have examined the connection between large igneous provinces of India (LIPs) and emplaced mafic dyke swarms in shield regions, particularly in contemporary research (Ernst, 2010). The archean Dharwar Craton is said to have been invaded by numerous distinct Palaeoproterozoic mafic dykes (Murthy, 1987, Drury, 1984; Halls, 1982).Comprehensive research has been conducted on the petrology and geochemistry of these palaeoproterozoic mafic dykes especially on those located in the eastern Dharwar Craton (EDC) (Chatterjee & Bhattacharji, 2001; Radhakrishana et al., 2007; Srivastava, et al, 2014).Comparable research data are known for the Western Dharwar Craton (WDC) (Devaraju 2008; Naqvi et al., 1972; Radhakrishna, 2009); nevertheless, a thorough investigation throughout the Indian peninsula is necessary due to the probable relevance of dykes in the crustal evolution. Therefore, a few mafic dykes exposed in the Mysore district of WDC, particularly in and around Piriyapatna talukregions (see Fig 1), have been sampled for preliminarypetrological and geochemical studiesinorder to understand its genesis and classification

## Regional Geology

Intruding all earlier stratigraphic formations from the Archean (>3.4 Ga) to the early-middle Palaeocene (0.6 Ga), dyke formations are found throughout Peninsular India and have a well-documented crustal evolution history (Murthy, 1995; Murthy, 1987, Ikramuddin&Steuber 1976;Ernst and Srivastava, 2008).However, the focus of this investigation is on dykes that are unique to the Piriyapatna taluk of Mysore district. Since these dykes are stratigraphically part of the southern WDC, the Dharwar Craton is briefly described below.

Dharwar craton is considered as greatest Archean continental nucleus in the Asian continent.This supergroup has a basement of tonalite–trondjemite–granodiorite (4.0–2.56 Ga) (TTG) gneisses, the amphibolite to granulite metamorphic facies (3.40–2.56 Ga) (Naqvi and Rogers, 1987; Peucat et al., 1995) and two generations of greenstone belts—the 3.35–3.20 Ga volcano sedimentary Sargur group and the low to medium grade metamorphic sequence (2.90–2.54 Ga) of Dharwarsupergroup (Radhakrishna, 1988; Radhakrishna, 1983). The EDC and WDC are the two main divisions of the Dharwar Craton, which are based on different geological aspects; such as lithological similarities, geochronological aspects and other structural geology characteristics.(Ramakrishnan, 1988, 1993; Ramakrishnan & Vaidyanadhan, 2010).The Chitradurga fault zone divides the EDC from the WDC. Recently, a central block that divides the craton into Western (3.4 Ga to 3.0 Ga crust), Central (>3.0 Ga crust), and Eastern blocks (2.7 Ga to 2.6 Ga crust) has also been postulated (Jayananda et al., 2018). According to the tectonic evolution history of the Dharwar craton, cratonization processes persisted until 2.5 Ga, after which there was a significant change in the petrogenetic and crustal development processes toward the late Archean (Manikyamba et al., 2004). These differences can be associated with a number of magmatic events which includes mafic dyke intrusion into basement rock formations such granitoids, TTGs, and greenstone belts.

In contrast to the mafic dykes in the EDC, those in the WDC differ in composition and orientation.According to Radhakrishna and Mathew (1993), research has revealed three primary dyke swarms in the WDC that are thought to be of Proterozoic age: (a) the Hassan–Tiptur dykes; (b) the Mysore dykes; and (c) the Dharwar dykes. A wider distribution of dolerite dykes and two suites of amphibolitic and epidioriticdyke swarms were suggested by Meert and Pandit (2015). Dyke swarms in the WDCare different from those in the Eastern Dharwar Craton in that they occur as patches of large exposures, most of which have no obvious relationship to the cooled margins and country rock. Additionally, these dykes are sporadically spaced and largely unreachable because of the deep forests that encircle a large portion of western Dharwar's north and the vegetation that covers the green stones.

### Field investigation and sampling

The Piriyapatna area of Mysore is selected due to the presence of a cluster of dykes of nearly similar trend (Fig. 1). In this location, there are exposed dykes of varying sizes, but not clearly showing contact with the country rock. Many of the mafic dykes are found partially destroyed in the middle of farmed land, while the majority are invaded as a result of agricultural activity. In this study area, dolerite dykes exhibit NNW-SSE trending intrusion embedded in the

PGC. The Piriyapatna dolerite dykes rocks range in color from dark green to black. Samples are collected from the exposed outcrops, and preliminary petrographical research and geochemical analysis were carried out to understand the geochemical characterisation and textural features of these dolerite dykes.

**Petrography:**

The mineral distribution, texture, crystallization and degree of alteration showed two distinct types of dykes from Piriyapatna taluk in the thinsection studies. The ophitic texture, which is characterized by lath-shaped plagioclase feldspar encased in a matrix of coarse grained pyroxene minerals, is shown in Fig. 2aand 2b shows the dolerite with medium-grained sub-ophitic texture and also which includes opaque minerals like magnetite and titanomagnetite in their dispersed forms, as well as laths of plagioclase feldspar and clino-pyroxene as essential minerals. Even though Fig. 2bhas a texture comparable to 2a, the presence of chlorite crystals in this area points to changes that this dyke rock has undergone. The Photomicrograph(Fig 2c.) showspoikilitictexture.The differing rates at which the minerals nucleate and grow typically result in a poikilitic texture; the slower-growing mineral, known as an oikocryst, encloses the rapidly-growing minerals, which stay as smaller grains. Because of the prominent metamorphic characteristics, these dyke rocks are classified as meta-dolerites. Plagioclase laths were maintained in 50% or fewer of the doleritic textures that relicts and amphibole had mostly replaced pyroxene. As the study area belongs to a lower degree metamorphic zone, the presence of the chlorite is convincing this.A few samples contained olivine in small quantity as well. Photomicrograph taken in a reflected microscope(Fig 2d) shows the presence of pyrite.Numerous reports of dolerite dykes with comparable petrographic characteristics may be found all around the WDC. (Halls 2007)

Fig 2: The Photomicrograph images of the dolerite dykes from study area. Fig (a) and (b) shows ophitic-subophitic texture with lath-shaped plagioclase in a pyroxene matrix; Fig (c) shows a poikilitic texture with a large plagioclase feldspar (oikocryst) where smaller pyroxene minerals are enveloped in plagioclase. Fig (d) shows Pyrite mineralization in a dolerite dyke.Amphibole is also present in these dykes, as the evidence for alteration.
Pl=Plagioclase,Cpx=Clino-pyroxene,Chl=Chlorite, Amp=Amphibole, Qz=Quartz, Py=Pyrite

**Analytical Techniques:**

**Whole-rock geochemistry**

For the dyke rocks in this work, a complete geochemical investigation employing XRF was carried out in order to provide a preliminary geochemical characterization. Whole rock analysis has been conducted on representative samples that were gathered. Steel and agate mortars are used to chip and grind samples till they are powdered to 63μ. Eight new samples were chosen for major oxide studies from the NE–SW trending Piriyapatna dykes.The XRF analysis is done at the National Centre for Polar and Ocean Research (NCPOR), and instrument used for this study isAxios Max WD XRF Spectrometry WDXRF spectrometer . Table 2 presents the findings. Major element concentrations differ with $SiO_2$ ranging from 47–50wt% whereas MgO and $Al_2O_3$ show very large differences, 7–14wt%, and 9–15wt%, respectively. The major oxides like $Fe_2O_3$ and CaO, shows variations 12-13 wt% and 9-11 wt% respectively. Other alkalies shows smaller variations.

## RESULTS AND DISCUSSION

The dolerite dykes are plotted in the basalt and basalt andesitic field, which essentially depicts the mafic nature of the rock, according to the total alkali against $SiO_2$ -TAS diagram (Fig. 3). The mafic index character of the dolerite dyke from the research area was determined using the Harker binary diagram. This is based on the MgO vs. Major oxides (Fig. 4) plot.As the MgO increases with decreasing $SiO_2$, the bivariate plot of MgOvs $SiO_2$ exhibits a negative correlation, which is consistent with normal magmatic crystallization patterns. There is also a negative association seen in the MgO vs. $Al_2O_3$, $K_2O$, and $TiO_2$ bivariate plot. In contrast, there is a positive correlation between MgO and $P_2O_5$ and $FeO^T$. The fractionation of Cpx during crystallization is clearly indicated by the positive correlation existing between CaO and MgO.

The AFM (A=$Na_2O+K_2O$; F=$Fe_2O_3^T$; M=MgO) plot of Irvine and Baragar (1971) diagram (Fig. 5a) for dolerite, and meta-dolerite rocks plot in tholeiitic field, and the both rocks shows distinct ferro-magnesium-enrichment trend. The Jensen (1976) triangular cation plot (Fig.5b) for dolerites plots more in Komatiticbaslt field while three of the samples falls at high-Mg tholeiite basalt field . The Classification of Miyashiro (1974) dividing the calc-alkaline series from the tholeiitic series, by plotting SiO2 VsFeO/MgO diagram (Fig. 6) applied for dolerite samples. The plot shows samples fall in both calc-alkaline series and tholeiite series, but most of the dolerites falls under tholeiite category. Several tectonic setting diagrams were used to denote the dolerite dyke emplacement characteristics. The ternary diagram (Fig.7) of Pearce et al.(1977) shows the plot of major oxides MgO-$Fe_2O_3^T$-$Al_2O_3$, the dolerite samples plot in Ocean Island and Ocean ridge floor fields.

The geochemical analysis of the dyke samples that were gathered in the current research highlights the following factors. Every dyke rock sample collected from the piriyapatna exhibits a doleritic composition; the main minerals found in these rocks are the Cpx and plagioclase feldspar, which have a lath shape, along with a few opaque minerals like magnetite and pyrites. The dolerite rock that has been gathered exhibits mostly subophitic textures, but few dykes shows poikilitictextures , and sulfide mineralization in a few more. The dolerite dykes from Piriyapatna intrudes into the Peninsular Gneissic Complex (PGC) like rest of the dykes from the entire Mysore. All of the dyke samples fall into the tholeiite to basaltic categorization fields based on the whole rock geochemical data.

## CONCLUSION

Based on its mineralogical, textural, and geochemical properties, the collected dyke rocks from the study area are categorized into dolerite dykes and meta-dolerites. Because the gathered dyke samples have a doleritic composition and contain a significant amount of Cpx and lath-shaped plagioclase as mineralogy. Thin section investigations also reveal the existence of alterations leading to the formation of amphiboles and chlorite minerals.A small number of dolerite dykes have sulfide mineralization, such as pyrite crystals, which is similar to many other WDC dykes.Subophitic textures can be seen in the gathered dolerite rock, and gabbroic textures with remnant doleritic intergrowth can be seen in a few dykes. Geochemically, these dolerites exhibit ferro-magnesium enrichment and tholeiite character.

The composition of dolerites can be seen as basaltic to basaltic andesitic based on the TAS plot. The MgO and compatible elements have a favorablecorrelation.The current dolerite samples' tectonic settings reveal features of oceanic ridges as well as Island Arc Basalt.This indicates that the dolerites with OIB and E-MORB affinities were most likely deposited in the oceanic ridge and transitional island arc environments.

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**Table 1: Displays the sample number and location, rock name, Lat/long, trend, and dyke texture.**

| Sl.No. | Sample No. | Latitude | Longitude | Trend | Rock name | Texture |
|---|---|---|---|---|---|---|
| 1 | N04 | 12° 25' 12.848'' N | 76° 6' 3.980'' E | N-S | Meta-dolerite | Poikilitic |
| 2 | N05 | 12° 26' 10.356'' N | 76°7 ' 5.648'' E | N-S | Meta-dolerite | Poikilitic |
| 3 | N06 | 12° 26' 0.888'' N | 76° 7' 10.690'' E | NNW-SSE | Dolerite | Sub ophitic |
| 4 | N07 | 12° 26' 11.161'' N | 76°9 ' 1.851'' E | NNW-SSE | Dolerite | Ophitic |
| 5 | N08 | 12° 21' 8.376'' N | 76° 10' 0.467'' E | NNW-SSE | Dolerite | Ophitic |
| 6 | N041 | 12° 20' 7.386'' N | 76° 8' 47.732'' E | NNW-SSE | Dolerite | Ophitic |
| 7 | N042 | 12° 20' 12.762'' N | 76° 7' 12.995'' E | NNW-SSE | Dolerite | Ophitic |
| 8 | N043 | 12° 19' 29.616'' N | 76° 4' 35.763'' E | NNW-SSE | Dolerite | Sub ophitic |

**Table 2. Whole- rock geochemistry for dolerite dyke rocks sampled from Piryapatna taluk, Mysore district, Karnataka.**

| Sample No. | N05 | N06 | N07 | N043 | N08 | N04 | N041 | N042 |
|---|---|---|---|---|---|---|---|---|
| (wt%) | | | | | | | | |
| $SiO_2$ | 47.99 | 49.16 | 51.62 | 49.15 | 49.21 | 50.94 | 48.23 | 48.33 |
| $TiO_2$ | 0.97 | 0.82 | 0.75 | 0.73 | 0.73 | 1.17 | 1.23 | 0.98 |
| $Al_2O_3$ | 14.59 | 15.15 | 13.87 | 9.53 | 9.55 | 13.65 | 12.32 | 11.46 |
| $Fe_2O_3$ | 11.35 | 12.17 | 10.65 | 13.46 | 12.94 | 11.56 | 12.9 | 13.45 |
| MnO | 0.17 | 0.16 | 0.16 | 0.18 | 0.18 | 0.14 | 0.18 | 0.19 |
| MgO | 12.02 | 8.93 | 8.85 | 13.68 | 14.7 | 7.72 | 12.09 | 12.22 |
| CaO | 9.76 | 8.92 | 10.48 | 9.31 | 9.33 | 11.16 | 9.4 | 10.14 |
| $Na_2O$ | 1.86 | 2.17 | 1.78 | 1.58 | 1.61 | 2.54 | 2.84 | 2.6 |
| $K_2O$ | 0.38 | 0.86 | 0.64 | 0.22 | 0.22 | 0.32 | 0.51 | 0.42 |
| $P_2O_5$ | 0.08 | 0.15 | 0.13 | 0.15 | 0.16 | 0.23 | 0.1 | 0.15 |
| LoI | 0.82 | 0.71 | 0.28 | 0.66 | 0.66 | 0.71 | 0.32 | 0.1 |
| Total | 100.00 | 99.20 | 99.21 | 98.65 | 99.28 | 100.13 | 100.13 | 100.04 |

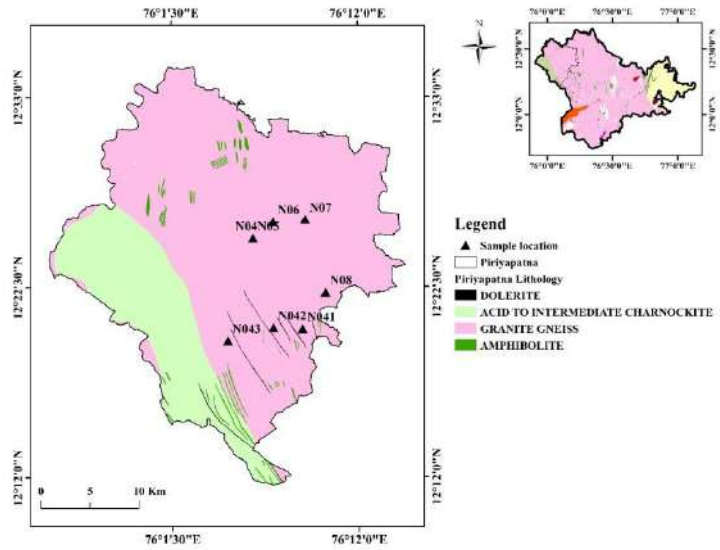


**Fig 1. The lithological map of the Piriyapatna Taluk including the dolerite dyke intrusion (represented as black line) and the geological map of the Mysore district is also shown at the top right corner. The sample locations are denoted in triangles. These dolerite dyke intrusions predominantly show NNW-SSE trends.**

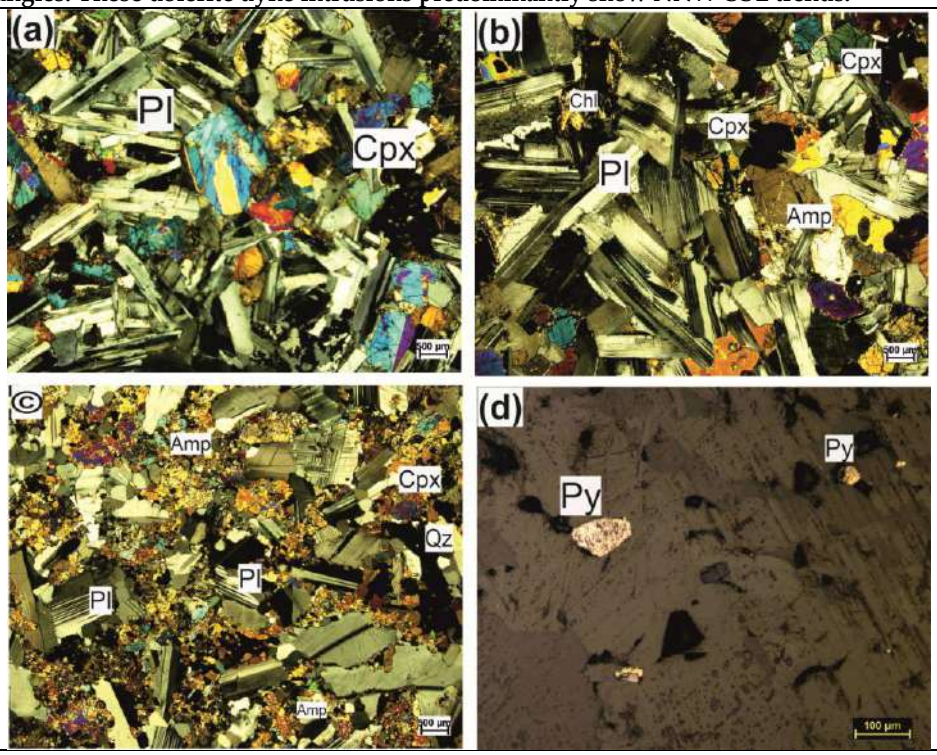


**Fig 2: The Photomicrograph images of the dolerite dykes from study area. Fig (a) and (b) shows ophitic-subophitic texture with lath-shaped plagioclase in a pyroxene matrix; Fig (c) shows a poikilitic texture with a large plagioclase feldspar (oikocryst) where smaller pyroxene minerals are enveloped in plagioclase. Fig (d) shows Pyrite mineralization in a dolerite dyke.Amphibole is also present in these dykes, as the evidence for alteration.**

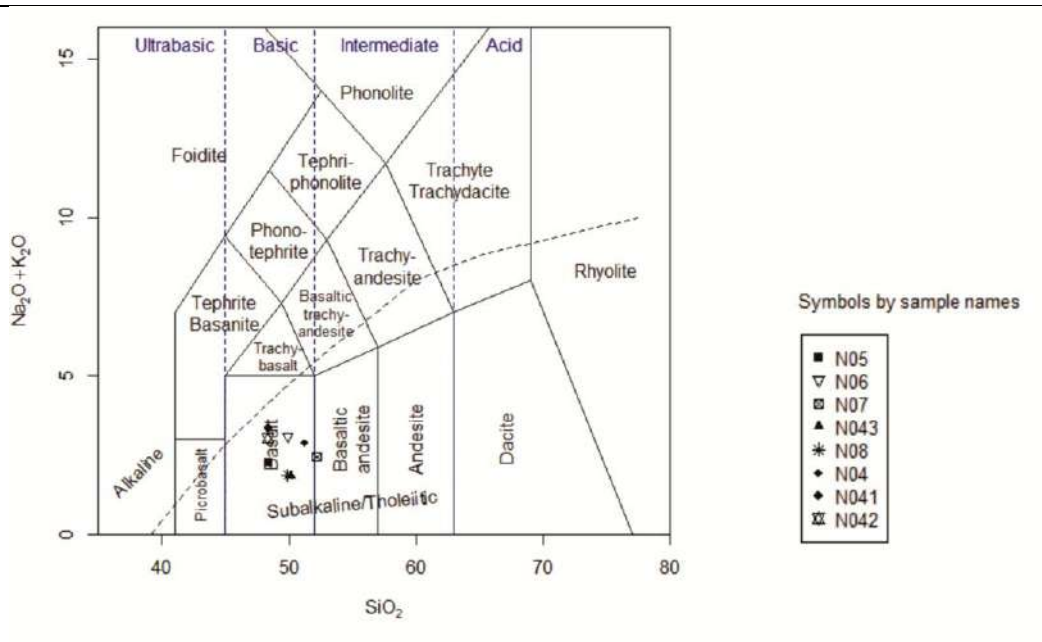


**Fig.3. From total Alkali ($Na_2O$ +$K_2O$)vs Silica (TAS) plot of Le Bas et al (1986), the general classification of collected dykes samples shows sub-alkaline tholeiitic in composition.The symbols which used to represent the sample name in this diagram is used in all other following diagrams.**

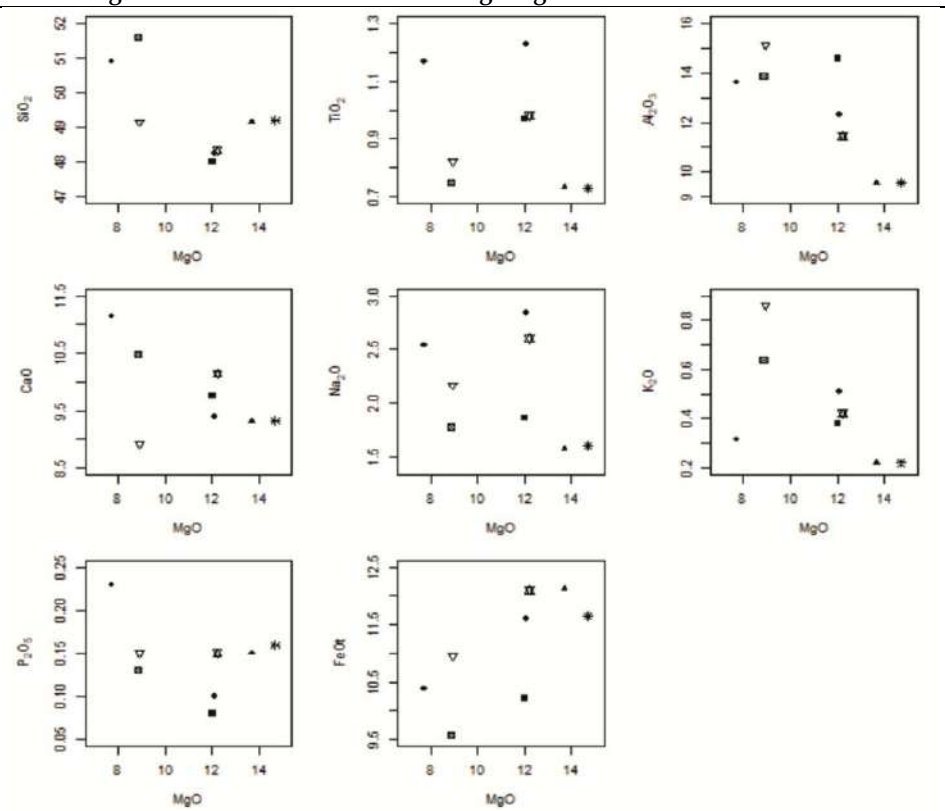


**Fig. 4: The Harker variation diagram MgO vs major elements.**

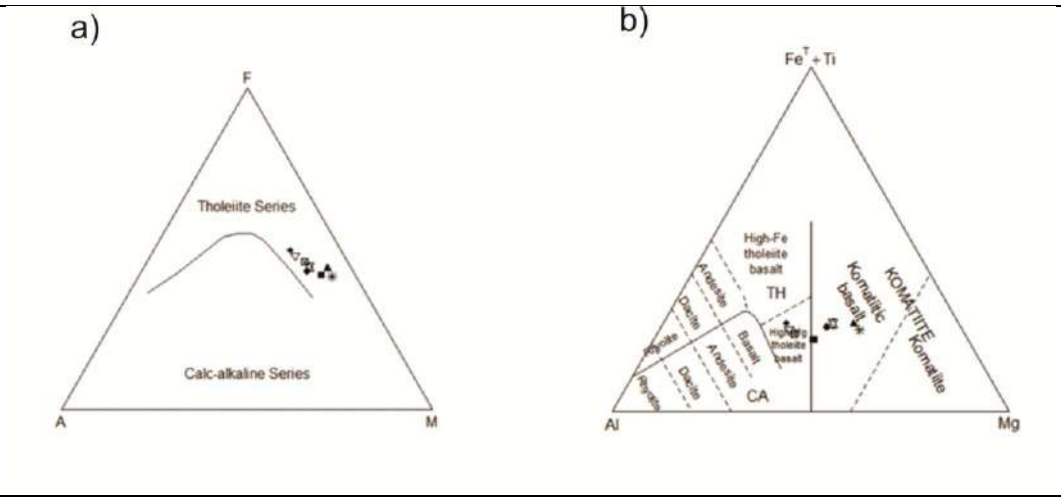


**Fig.5 a) The tholeiitic nature of the chosen samples is depicted in the Irvine and Baragar - AFM diagram (1971), and b) Jensen's cation plot (Jensen, 1976).**

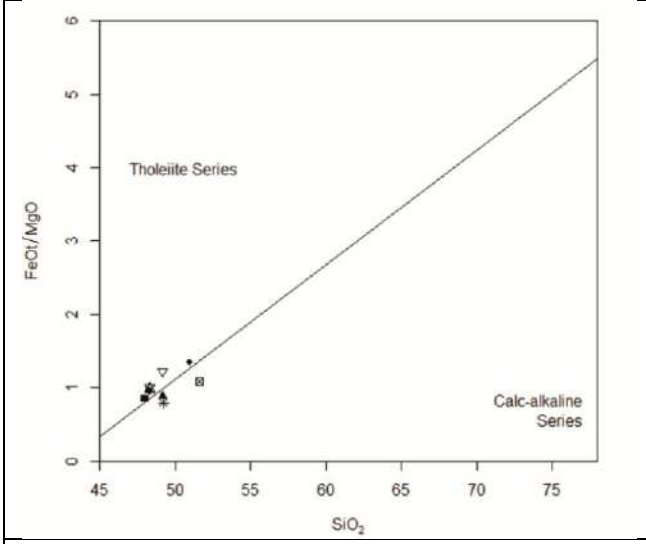


**Fig.6: The bivariate classification diagram, $SiO_2$ vs $FeO^T/MgO$ dicrimination plot for the calc-alkaline and tholeiite series after Miyashiro (1974).**

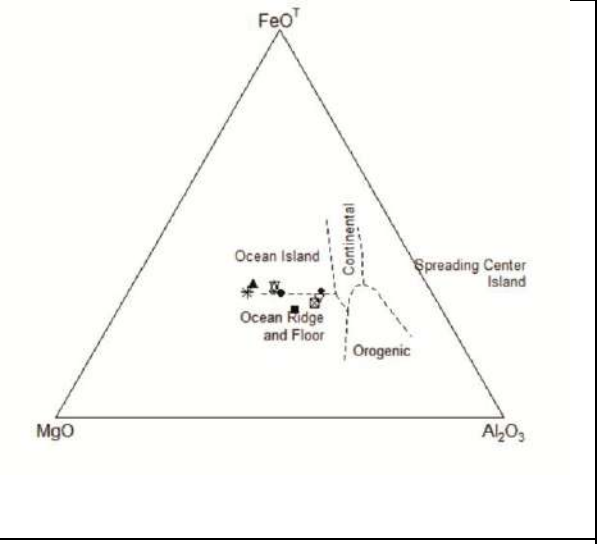


**Fig.7: The ternary diagram of Pearce et al. (1977) using the major oxides $MgO$-$Fe_2O_3^T$-$Al_2O_3$**

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**REVIEW ARTICLE**

# HPTLC Method Validation of Antidiabetic Drugs - A Short Review

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## ABSTRACT

One essential analytical technique for measuring and characterising anti-diabetic medications is High-Performance Thin-Layer Chromatography (HPTLC). Since diabetes mellitus is becoming an epidemic on a worldwide scale, precise medication analysis is now essential. Among the many benefits of HPTLC are its great sensitivity, cost-effectiveness, and repeatability. The fact that diabetes is a major global health concern highlights how crucial accurate medication analysis is to the treatment of the condition. HPTLC is a great option for this task because of its exceptional accuracy in separating and quantifying complicated drug combinations. Sensitive detection methods, strict chromatographic conditions, and careful sample preparation are standard components of HPTLC methodologies for antidiabetic medication analysis. To guarantee accurate outcomes, issues like validation and standardization of methods are acknowledged and resolved. Applications for HPTLC in antidiabetic medication analysis include quality control, pharmacokinetic research, and study of herbal medicines. Its adaptability to various medication formulations and sample types increases its usefulness for diabetes management research and practice. HPTLC is a reliable analytical method for characterising and quantifying medications that treat diabetes. To improve HPTLC techniques and increase its use in diabetes research and therapy, more investigation and development are necessary.

**Keywords:** HPTLC, antidiabetic, development, analysis.

## INTRODUCTION

Diabetes mellitus is a group of metabolic conditions characterized by chronically elevated blood sugar levels due to defects in the production or function of insulin, or both. Insulin, being a crucial hormone for anabolism, causing disruption in the consumption of proteins, lipids, and carbohydrates. The insufficient insulin to create deduce response, insulin resistance of target tissues, liver, skeletal muscles and adipose tissue can be the causes of these metabolism abnormalities. Insulin receptors, the signal transduction system, effector enzymes, and genes are the sites of injury. The kind and duration of diabetes determine how severe the symptoms are[1,2].Diabetes mellitus (DM), the most common endocrine disorder, is expected to impact more than 200 million people worldwide by 2010 and an additional 300 million by 2025. As the disease deteriorates, there is a progression of tissue or vascular damage, leading to severe consequences associated with diabetes, such as ulceration, neuropathy, retinopathy, and nephropathy [3]. The American Diabetes Association (ADA) continues to employ the most universally acknowledged classification of diabetes, which comprises type 1, type 2, various variants, and gestational diabetes mellitus (GDM). The ADA initially introduced this categorization in 1997 [4].Diabetes Mellitus Type 1 (T1DM) caused by reduced level of beta cells which produce insulin. These beta cells are demolished by immune system. Usually takes place in childhood or adolescence, while it can happen at any age. Treatment includes lifelong insulin medication, lifestyle modification, and blood sugar monitoring[5].Diabetes Mellitus Type 2 (T2DM),The reason behind T2DM Insulin deficit relative to insulin resistance. Most often affects persons over 45, although as obesity rates rise, it is also becoming more prevalent in younger populations. Prevention of T2DM includes handling alterations in lifestyle (food, exercise), oral drugs, and occasionally insulin[6].GDM, or gestational diabetes mellitus caused by pregnancy-related hormonal changes that result in insulin resistance. occurs during pregnancy and normally goes away after birth, although it raises the possibility of acquiring type 2 diabetes in the future. Exercise, dietary changes, and occasionally insulin or oral medicines are used as treatments[7].Additional Particular Types: Monogenic diabetes, which includes MODY (Maturity Onset Diabetes of the Young), is brought on by mutations inasinglegene. Secondary diabetes: Few drugs might cause side effects , as a result of pancreatic disease, or as a result of hormonal imbalances. All forms of diabetes must be managed by routinely checking blood glucose levels, leading a healthy lifestyle, and following recommended treatment plans. In order to avoid complications including cardiovascular disease, nerve damage, kidney failure, and eye issues, early diagnosis and treatment are essential[8].

Medication used to control diabetes mellitus that lowers blood glucose levels is called an antidiabetic drug. Important varieties and their mechanism of action (MOA) includes: Insulin-Helps cells absorb glucose by replacing or enhancing endogenous insulin, especially in type 1 diabetes. Metformin-A biguanide that enhances peripheral tissue insulin sensitivity while lowering the amount of glucose produced by the liver. Sulfonylureas- Promote the release of more insulin from pancreatic beta cells (e.g., glipizide). DPP-4 Inhibitors- Raise incretin levels, improving insulin secretion and lowering glucagon release (e.g., sitagliptin). GLP-1 Receptor Agonists- mimic incretin, raise insulin release, lower glucagon secretion, and decrease stomach emptying (e.g., exenatide).SGLT2 Inhibitors- These medications, such as Empagliflozin, prevent the kidneys from reabsorbing glucose, which encourages urine excretion of glucose. Thiazolidinediones, such as pioglitazone, increase muscle and adipose tissue's sensitivity to insulin by stimulating PPAR-gamma receptors. Alpha-Glucosidase Inhibitors (e.g., acarbose)- Reduce postprandial glucose increases by delaying the intestines' absorption of carbohydrates. The kind of diabetes, comorbidities, and specific patient needs are taken into consideration when choosing these drugs[9-11].

Chromatography is a method used to separate the different elements of a mixture, known as solutes, based on their relative amounts in a moving fluid stream (called the mobile phase) and a stationary phase that is continuous. The stationary phase can exist as either a solid or a liquid, whereas the mobile phase can exist as either a liquid or a gas. Chromatography is extensively employed in the fields of chemistry and biology. In biochemical research, it is often used to detect and separate chemical compounds that originate from biological sources. This approach is employed in the petroleum industry to analyse complex hydrocarbon blends [12,13].

**Significant categories consist of**

1. TLC, or thin-layer chromatography: Mechanism- By using capillary action to transfer a solvent (mobile phase) along the glass plate, a sample is applied to a stationary phase (often silica gel) and separated. Use- Tracking the development of reactions and qualitatively analysing substances[14].
2. High-Performance Liquid Chromatography (HPLC): Mechanism- Under high pressure, a sample is injected into a liquid mobile phase and passes through a stationary phase-containing column, separating components according to how they interact with the stationary phase. Application- Both quantitative and qualitative examination of intricate blends in food, medicine, and environmental specimens[15].
3. Gas Chromatography (GC): Mechanism- To separate components based on volatility and interactions with the stationary phase, a volatile sample is vaporized and transported by an inert gas (mobile phase) along a column containing a liquid or solid stationary phase. Use- Analysis of volatile chemicals, for example, in food analysis and environmental monitoring[16].
4. Ion Exchange Chromatography: Mechanism- Ions and polar molecules are separated according to how well they cling to ion exchangers in the stationary phase. Use- Water analysis, protein purification[17].
5. SEC, or size-exclusion chromatography: Mechanism- Smaller molecules travel longer routes and elute later; larger molecules are separated according to size by passing them through a column loaded with porous beads. Use- Analysis of polymers and proteins[18].
6. High-Performance Thin Layer Chromatography:Better resolution and sensitivity are ensured by using tiny particle size stationary phase and high-quality, repeatable pre-coated plates. Samples are carefully applied as tiny spots or bands in HPTLC, and components are separated when the mobile phase (solvent) rises up the plate due to capillary action. Sophisticated scanning densitometers or other detection systems are used for detection and quantification; these devices are frequently combined with data analysis software. Because HPTLC is efficient, accurate, and repeatable, it is frequently used for both qualitative and quantitative analysis in the food, pharmaceutical, and environmental testing industries, as well as in the examination of plant materials[19,20].

**High Performance Thin Layer Chromatography (HPTLC)**

High Performance Thin Layer Chromatography is an advanced chromatographic technique used for the separation, identification, and quantification of chemical compounds in complex mixtures. Similar to traditional thin layer chromatography, HPTLC involves the separation of components based on their differential migration rates through a thin layer of stationary phase on a glass or plastic plate. However, HPTLC employs finer stationary phases, improved instrumentation, and optimized conditions, resulting in higher resolution, sensitivity, and reproducibility compared to conventional TLC. HPTLC is widely utilized in various industries, including pharmaceuticals, food, cosmetics, and environmental analysis, due to its versatility, speed, and cost-effectiveness. It plays a crucial role in quality control, research, and regulatory compliance by facilitating the analysis of diverse sample types with minimal sample preparation requirements [21-22.]

**Principle**

The principle of HPTLC involves separating compounds based on the rate of solvent migration on the thin layer of stationary phase, typically silica gel or cellulose, through capillary action. This separation occurs due to differences in the affinity of the compounds for the stationary phase and the mobile phase, which moves up the plate by capillary action[23]. The separated compounds are then visualized and analysed using various detection methods, such as UV absorption or chemical staining.

**Advantages**

1. High resolution: It can separate compounds with similar properties effectively, providing better resolution compared to traditional TLC.
2. High sensitivity: HPTLC allows for the detection of even trace amounts of compounds, making it suitable for quantitative analysis.
3. Speed: HPTLC provides faster analysis times compared to other chromatographic techniques, allowing for high throughput analysis.

4. Versatility: It can analyse a wide range of compounds, including pharmaceuticals, natural products, and food additives[24].
5. Cost-effectiveness: HPTLC typically requires less solvent and sample compared to other chromatographic techniques, making it more cost-effective.
6. Ease of use: HPTLC systems are relatively simple to set up and operate, requiring minimal training for users.
7. Quantitative analysis: HPTLC can be used for both qualitative and quantitative analysis, providing accurate measurements of compound concentrations[25].

**DISADVANTAGES**

While HPTLC offers many advantages, it also has some limitations:

1. Sensitivity: Although HPTLC can detect trace amounts of compounds, its sensitivity may not be as high as other chromatographic techniques like HPLC or GC-MS.
2. Limited compound compatibility: Some compounds may not separate well on HPTLC plates due to interactions with the stationary phase or mobile phase, limiting its applicability for certain analyses[26].
3. Sample preparation: HPTLC requires many steps including extraction and concentration, which might take more time and increased worker need.
4. Lack of automation: Compared to other chromatographic techniques, HPTLC systems may have limited automation capabilities, leading to potential variability in results and increased operator dependence.
5. Plate uniformity: The uniformity of HPTLC plates can vary, leading to potential inconsistencies in separation and detection.
6. Quantitative limitations: While HPTLC can provide quantitative results, it may not be as precise or accurate as other quantitative techniques like HPLC or GC-MS.
7. Limited detection options: HPTLC typically relies on visual or UV detection methods, which may not be suitable for all compounds or applications requiring specific detection wavelengths or sensitivity levels[27].

**Applications**

High-Performance Thin Layer Chromatography, or HPTLC, is a commonly used analytical method in many industries, but especially in chemistry and medicine. Among its uses are:

1. Quality control: HPTLC is used to examine and detect the presence of distinct components in a mixture, which assists in ensuring the quality of pharmaceutical items, food, and other consumer goods.
2. Drug analysis: To support forensic investigations, HPTLC is used to detect and quantify a wide range of drugs, including stimulants, opioids, and other restricted compounds.
3. Research in the pharmaceutical industry: To ensure the creation of safe and efficient medications, researchers employ HPTLC to examine the synthesis, pureness, and deterioration of pharmaceutical chemicals.
4. Environmental analysis: HPTLC is used in soil, water, and air samples to identify and quantify contaminants, including heavy metals and pesticides, which aids the process of environmental monitoring and assessment.
5. Food analysis: To ensure the safety and quality of food, HPTLC is used in the identification and determination of multiple food additives, pollutants, and integrity testing of food products[28].
6. Plant research: HPTLC is utilized in the detection and measurement of bioactive chemicals in plants, assisting in the development of new medications as well as comprehending plant metabolism.
7. Biological samples analysis: HPTLC is employed in the analysis of biological samples, such as urine, blood, and tissues, for the detection of drugs, toxins, and other biomolecules.
8. Counterfeit detection: HPTLC can be used to detect counterfeit products in various industries, such as pharmaceuticals, cosmetics, and consumer goods, by analysing their composition and authenticating their origin[29].

**Comparison of TLC and HPTLC**

TLC and HPTLC are based on the same principle i.e. adsorption. The comparison between TLC and HPTLC is listed below.

**HPTLC Instrumentation**

**Sample Applicator**

The HPTLC- Automatic Applicator AS30 samples are sprayed onto TLC/HPTLC plates with compressed air flow without contacting the plates. The sample Application is carried out automatically, only filling and inserting the syringe is made manually. The dosing syringe is fastened at flexible turret, thus detaching and calibrating of syringe is not necessary. It can be filled either manually by the filling syringe or by the Autosampler BS35 (Optional). This is remarkable and time-saving particularly when large sample throughput is present[33]. Up to 80 different samples can be appliedcompletely automatically. The spray on technique is beneficial for quantitative analysis as it is possible to have different volumes of same calibration standard instead of equalvolumes of different standard concentrations. Even if samples are dissolvedin polar solvents such as water, compact and narrow bands are formed.

Key Aspects of HPTLC-Applicator AS30

- Spray-on technique
- Dual syringe system: dosing and filling syringe
- Application of dots or lines
- 2D application
- For any TLC/HPTLC plates, dimensions= 200 x 200 mm
- Stand-alone or PC-controlled
- Holding up to 10 methods
- Low sample requirement
- Incl. software for controlling AS30/BS35 and
- Transferring data to Densitometer CD60
- Special cover for active exhaustion on request[34]

Technical Parameters of HPTLC-Applicator AS30

- Plate size = 200 x 200 mm
- Path length 0 – 100 mm in steps of 0.1 mm
- dosing syringe= 10 µl,
- filling syringe= 25 µl
- Application rate= 3 – 120 s/µL
- Resolution= 2000 steps/µL
- Turret movement= 40 mm/s, 13 steps/mm
- Necessary air pressure= 2.5 – 10 bar [35-36]

**Uses of sample applicator:**

The software manages the sample container selection, transport, and washing procedures using a serial interface.

**TLC Scanner**

The TLC Scanner 4 is a very sophisticated workstation designed for precise examination of TLC/HPTLC chromatograms and other planar objects using densitometric methods. The TLC Scanner 4 is a device used for measuring the density of scanned images. The process involves quantifying the reflectance of individual substances using absorption and/or fluorescence methods[37].

**Uses**

A Thin Layer Chromatography (TLC) scanner is an apparatus specifically intended to assess the radiochemical purity of certain radiopharmaceuticals, which are substances utilized as dyes or medicines in nuclear medicine procedures including bone scans and PET CT scans. In order to detect natural products such as essential oils, volatile oils, fixed oils, glycosides, waxes, alkaloids, and so on[38]. It is commonly employed in the separation of complex medicinal compositions including many components.

**Chromatogram**

High-performance thin-layer chromatography (HPTLC) is an advanced version of thin-layer chromatography (TLC) that provides enhanced strength, ease, quickness, and effectiveness in quantitatively analysing substances. This thin-layer chromatography (TLC) analytical method improves the separation of compounds, resulting in more accurate quantitative measurement[39]. The HPTLC chamber is pre-conditioned with solvent to ensure the presence of consistent solvent vapours throughout the chamber. There are four methods for developing chromatograms. The four methods are: 1) Vertical method, 2) Vario-method, 3) Horizontal method, and 4) Automatic multiple development.

**Purpose of Chromatogram**

Chromatography is a significant biometric technique which is used to separate, identify, and purify the chemical compounds from mixture of compounds at qualitative and quantitative level.Chromatogram growth, which is the separation process in the HPTLC technique, is often disregarded despite its critical importance. Key parameters associated with this stage are typically neglected. HPTLC plates are generated using twin-trough chambers or horizontal-development chambers. Typically, twin-trough chambers that are saturated and equipped with filter paper provide the highest level of repeatability. The twin-through chamber prevents the preloading of solvent vapor and humidity. The detection of individual chemicals on the sorbent layers is improved by the suppression of fluorescence caused by ultraviolet (UV) light, typically in the range of 200-400 nm. This phenomenon is frequently referred to as Fluorescence quenching[40]. The visualization at UV 254 nm F254 can be accurately defined as phosphorescence quenching. In this case, the fluorescence persists briefly after the excitation source is no longer resent. It has a brief duration, yet exceeds 10 seconds. The F254 fluorescent indicator is stimulated by a UV wavelength of 254 nm and produces a green fluorescence. Compounds that exhibit absorption of light at a wavelength of 254 nm cause a decrease in the emission on the layer, resulting in the formation of a dark violet spot on a green background at the locations where the compound zones are present. The quenching phenomenon is a result of the presence of molecules with conjugated double bonds. Anthraglycosides, coumarins, flavonoids, propyl phenols found in essential oils, as well as alkaloids such as indole, isoquinoline, and quinoline alkaloids, need to be identified using a 254 nm wavelength. Fluorescence quenching is the appropriate term to describe the visualization at UV 366 nm F 366. In this case, the fluorescence does not persist once the excitation source is withdrawn. All anthraglycosides, coumarins, flavonoids, phenolcarboxylic acids, and certain alkaloid types (such as Rauwolfia and Ipecacuanha alkaloids) demonstrate this quenching effect. The presence of separated compounds in the white light Zone may be identified by observing their inherent hue under daylight conditions. Derivatization Derivatization is an approach procedure which modify the functionality of an analyte to facilitate chromatographic separations. Derivatization can be established by either immersion or spraying of the plates with an appropriate reagent.

**Spectrophotometer**

HPLC systems utilize a UV-detector to quantify concentration via absorbance spectroscopy. The concentration may be determined using the Beer-Lambert's Law equation: $A = \varepsilon c L$. The detector quantifies the degree of light absorption over time at one or more specific wavelengths.A novel technique for separation, utilizing High Performance Thin Layer Chromatography in combination with UV and fluorescence detection, allows for the differentiation of non-volatile hydrocarbons into distinct groups based on the number of aromatic rings present, ranging from two to five. This approach also distinguishes between condensed and non-condensed structures. The technique employs a two-step migration process using ethyl acetate/n-hexane and n-hexane solvents on RP-plates, maintained at a temperature of -23 °C. HPTLC is utilized for quantifying tar by the usual addition approach, which involves combining all the aromatic components into a single peak. Subsequently, two methods are suggested for measuring UV levels: the first method, known as UV direct quantification, involves estimating the absorbance and concentration of a known sample, which allows for the calculation of the concentration of an unknown sample[41]. The second method, utilizing UV spectra deconvolution (UVSD), quantifies the concentration of tar by performing UV deconvolution on specific standard spectra, eliminating the requirement for reference samples. The two methods were utilized on a total of thirty-seven samples obtained from three different gasifiers: a Bubbling Fluidized Bed

(BFB), an Internal Circulating Bubbling Fluidized Bed (ICBFB), and a Circulating FluidizedBed(CFB).

**Developed Plate**

A series of HPTLC plates were prepared with the solutions in the ratio of 4:1(volume/volume) using a mixture of methanol and water. The plates were filled with this mixture until it reached the top edge of the plate in the Simultan Separating Chamber (manufactured by biostep, Burkhardtsdorf, Germany). They were then dessicated in the oven at a temperature of 110 °C for a duration of 20 minutes. After drying, a clean counter glass plate was placed on top of the stacked plates, and the entire setup was wrapped in aluminium foil for storage in a desiccator. Due to the high cost of high purity standards, the application parameters were chosen to minimize the loss of microliter volume during the automated syringe operation. This was achieved by setting the filling vacuum time to 1 second, the rinsing vacuum time to 6 seconds, and the number of rinsing/filling cycles to 1. Additionally, any unused sample was returned to the vial using the Automatic TLC Sampler ATS4 by CAMAG in Muttenz, Switzerland. The process of drying the plate was consistently carried out using a cold air stream, either by a hair drier or the Automatic Developing Chamber 2 (ADC 2) by CAMAG. This was done immediately after the application of the substance (within 0.5 minutes) and the subsequent development (within 5 minutes). The ambient air had a relative humidity of around 40 ± 5% during the developments. If necessary, the humidity of the plate can be modified by use a saturated solution of sodium acetate[42]. The program visionCATS (version 3.0, CAMAG) was used to process all instrument operation and obtained data

**UV Detection**

An HPTLC technique with UV detection has been developed to assess the purity of synthesis products. The consistency between the measurements of purity obtained using HPTLC-UV and LC-UV indicates that the approach is appropriate for this application. Atmospheric-pressure matrix-assisted laser desorption/ionization mass spectrometry (AP-MALDI-MS) was employed to verify and determine the product regions on the plate prior to purification by preparative-layer chromatography (PLC). AP-MALDI-MS exhibited higher selectivity compared to UV detection and provided a convenient and quick method for identifying synthesis products directly on the plate. This article presents a novel and uncomplicated gadget designed for the purpose of isolating product zones that have been split by a programmable logic controller (PLC). By utilizing PLC (Preparative Liquid Chromatography) in conjunction with the latest isolation technology, the purification of synthesis products in quantities less than one milligram was accomplished with success [43].

**Antidiabetics Drugs - HPTLC Method Validation**

Anti-diabetic drugs validated by HPTLC method are mentioned in Table 2.

## CONCLUSION

The HPTLC (High-Performance Thin-Layer Chromatography) technology provides an efficient and accurate method to analyze medications that prevent diabetes. Its excellent consistency, sensitivity, and specificity make it an effective tool for quality assurance and pharmacological research. HPTLC plays a vital role in the development and assessment of anti-diabetic drugs by precisely separating and quantifying active components, which ultimately improves patient care and treatment results.Further, the versatility of the HPTLC approach enables the simultaneous study of several anti-diabetic medications, expediting the analytical procedure and conserving time and money. It may be used in laboratories with varying degrees of equipment and experience due to its adaptability to varied sample matrices and its very straightforward sample preparation procedures.

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**Table 1. Comparison of TLC and HPTLC**

| ASPECT | TLC | HPTLC |
|---|---|---|
| Full Form | Thin Layer Chromatography | High Performance Thin Layer Chromatography |
| Purpose | Separation and identification of compounds | Similar to TLC, but offers higher resolution and sensitivity[30] |
| Sample Size | Small | Small to medium-sized samples |
| Stationary Phase | Silica gel or alumina coated on a glass, plastic or aluminium plate | Same as TLC, but with optimized particle size and uniformity for improved separation[31] |
| Mobile Phase | Liquid | Liquid |
| Plate Size | Small<br>Dimensions = 20 cm x 20 cm | Larger<br>Dimensions = 20 cm x 20 cm or more |
| Resolution | Moderate | Higher |
| Sensitivity | Moderate | Higher |
| Speed of Analysis | Moderate | Faster |
| Quantitative Analysis | Possible but less accurate | More accurate |
| Instrumentation | Simple | Specialized equipment |
| Applications | Qualitative analysis, preliminary separation | Quantitative analysis, purity testing, quality control[32] |
| Cost | Lower | Higher |

**Table 2. Anti-diabetics drugs are validated by HPTLC method**

| ANTI DIABETICS DRUGS | HPTLC METHOD | VALIDATION PARAMETERS | REFERENCE |
|---|---|---|---|
| Glinate-MF (Nateglinide and metformin hydrochloride) | **Stationary phase:**Pre-coated silica gel 60 F-254 aluminium plates (10 · 10 cm, 250 cm thickness)<br>**Mobile phase:** Chloroform: Ethylacetate: Acetic acid<br>**Wavelength** = 216 nm | **Linearity range:**<br>Nateglinde: 200-2400 ng band$^{-1}$<br>Metformin hydrochloride: 500-3000ng band$^{-1}$<br>**LOD**<br>Nateglinde:0.020 ng/ band<br>Metformin hydrochloride: 0.022 ng/band<br>**LOQ**<br>Nateglinde:0.060 ng/band<br>Metformin hydrochloride: 0.066 ng/band<br>**Repeatability**<br>Nateglinde:0.725 %<br>Metformin hydrochloride: 0.730 %<br>**Intra-day Precision**<br>Nateglinde:0.710 %<br>Metformin hydrochloride: 0.361 %<br>**Inter-day Precision**<br>Nateglinde:0.790 %<br>Metformin hydrochloride: 0.837 % | 44 |
| Alogliptin benzoate | **Stationary phase:** Pre-coated silica gel 60F254 HPTLC plates (20 × 10 cm, layer thickness 0.2 mm) | **Linearity range**: 100- 5000 ng band$^{-1}$<br>**Repeatability**: 100.99%<br>**Intra-day Precision**: 1.17%<br>**Inter-day Precision**: 0.54% | 45 |

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| | **Mobile phase:** Acetonitrile: 1% Ammonium acetate in Methanol (4.5:5.5 v/v)<br>**Wavelength:** 277nm | **LOD:** 2.356 ng/band<br>**LOQ:** 7.14 ng/band<br>**Robustness:** Robust<br>**Specificity:** Specific<br>**Selectivity:** Selective | |
| Janumet (metformin hydrochloride and sitagliptin phosphate) | **Stationary phase:**Silica gel precoated aluminium plate 60F- 254 plates, (10 cm X 10 cm with 250 µm thickness)<br>**Mobile phase:**Acetone: Methanol:Toluene:Formic acid (4:3:2:1 v/v/v/v)<br>**Wavelength:** 220nm | **Linearity Range:**<br>Metformin hydrochloride: 2000-5000 ng band$^{-1}$<br>Sitagliptin phosphate: 200-500 ngband$^{-1}$<br>**Accuracy:**<br>Metformin hydrochloride: 100.19%<br>Sitagliptin phosphate:100. 47%<br>**Precision:**<br>**Intra-day Precision:**<br>Metformin hydrochloride: 41.74 %<br>Sitagliptin phosphate: 67.16 %<br>**Inter-day Precision:**<br>Metformin hydrochloride: 0.74 %<br>Sitagliptin phosphate:0.40 %<br>**LOD:**<br>Metformin hydrochloride: 45 ng/band<br>Sitagliptin phosphate:27 ng/band<br>**LOQ:**<br>Metformin hydrochloride: 150 ng/band<br>Sitagliptin phosphate:87 ng/band | 46 |
| Pioglitazone, metformin, and glimepiride | **Stationary phase:**Precoated silica gel 60 F254<br>**Mobile phase:**Acetonitrile:Methanol:Propyl alcohol: ammonium acetate 7:2:1:1 (v/v).<br>**Wavelength:** 240nm | **Linearity:**<br>Pioglitazone: 300-1200 ng band$^{-1}$<br>Metformin:10000-40000 ng band$^{-1}$<br>Glimepiride:40-160 ng band$^{-1}$<br>**Limit of Detection:**<br>Pioglitazone:56.57 ng<br>Metformin:6160.85 ng<br>Glimepiride:11.99 ng<br>**Limit of Quantitation:**<br>Pioglitazone:177.43 ng<br>Metformin:18669.26 ng<br>Glimepiride:36.33 ng<br>**Precision:**<br>**Intra-day Precision:**<br>Pioglitazone:0.4026 %<br>Metformin:0.4874 %<br>Glimepiride:0.2012%<br>**Inter-day Precision**:<br>Pioglitazone:0.5381 %<br>Metformin:0.4222 %<br>Glimepiride:0.5684 %<br>**Accuracy:**<br>Pioglitazone:1.215 %<br>Metformin:0.851 % | 47 |

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| | | Glimepiride:1.234 %<br>**Specificity:** specific | |
| Canagliflozin | **Stationary phase:** Precoated silica gel 60 F254 HPTLC aluminium plates (20 × 10 cm, 0.2mm thick).<br>**Mobile phase**: Toluene: Ethyl acetate: Methanol (2:2:1).<br>**Wavelength**: 290 nm. | **Linearity:** 10-500 ng/ band<br>**Intra-day Precision:** 0.01 - 0.2 %<br>**Inter-day Precision:** 0.01-0.2 %<br>**Recovery:** 99.04- 99.82%<br>**Limit of Detection:**0.39 ng/ band<br>**Limit of Quantification:** 1.19 ng/ band | 48 |
| Teneligliptin and Metformin | **Stationary phase:** Silica gel G60 F254 aluminium sheet (10x10 and 10x20 cm)<br>**Mobile phase**: Methanol: Ammonium sulphate: Triethylamine (9: 2.7: 0.5 v/v/v)<br>**Wavelength**: 237 nm. | **Linearity:**<br>Teneligliptin:4-28 ng/band<br>Metformin:100-700 ng/band<br>**Precision:**<br>**Intra-day Precision:**<br>Teneligliptin:0.82-1.01 %<br>Metformin: 0.93-1.15%<br>**Inter-day Precision:**<br>Teneligliptin: 1.06-1.07 %<br>Metformin: 1.19-1.27 %<br>**Accuracy:**<br>Teneligliptin: 0.12 %<br>Metformin: 0.49 %<br>**Limit of Detection:**<br>Teneligliptin:0.3 ng/spot<br>Metformin: 27 ng/spot<br>**Limit of Quantitation:**<br>Teneligliptin: 3 ng/spot<br>Metformin: 85 ng/spot<br>**Specificity:** Specific | 49 |
| Glynase-MF tablets (metformin hydrochloride and glipizide) | **Stationary phase:** Precoated silica gel 60 F254 TLC plates (10*10 cm)<br>**Mobile phase**: water: methanol: 0.5% w/v ammonium sulfate solution (6:3:1.5 v/v/v).<br>**Wavelength**: 236 nm. | **Linearity:**<br>Metformin hydrochloride:5000-25000 ng/band<br>Glipizide:50-250 ng/band<br>**Precision:**<br>**Intra-day precision:**<br>Metformin hydrochloride: 0.116%<br>Glipizide: 1.424%<br>**Inter-day precision:**<br>Metforminhydrochloride: 0.771%<br>Glipizide: 1.468%<br>**Accuracy:**<br>Metformin hydrochloride:99.9- 101.5 %<br>Glipizide:98.1-98.4%<br>**LOD:**<br>Metformin hydrochloride: 991.30ng/band<br>Glipizide: 9.57ng/band<br>**LOQ:** | 50 |

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| | | Metformin hydrochloride: 3003.95ng/band<br>Glipizide: 29.01ng/band<br>**Selectivity:**selective | |
| Triglucored tablets (Glibenclamid, Rosiglitazone maleate and Metformin Hydrochloride) | **Stationary phase:**Precoated RP-18 F254s TLC Aluminum sheets (20*10 cm)<br>**Mobile phase**: Methanol: Tetrahydrofuran: Water:Glacial acetic acid (16: 3.6: 4: 0.4 v/v)<br>**Wavelength**: Glibenclamide -237nm, Rosiglitazone maleate -324 nm Metformin hydrochloride – 240 nm | **Linearity:**<br>Glibenclamide: 200–1000 ng/band<br>Rosiglitazone maleate: 200–1000 ng/band<br>Metformin Hydrochloride: 120–600 ng/band<br>**Precision:**<br>**Intra-day precision:**<br>Glibenclamide: 0.44 %<br>Rosiglitazone maleate: 1.004 %<br>Metformin Hydrochloride: 0.362%<br>**Inter-day precision:**<br>Glibenclamide: 0.368 %<br>Rosiglitazone maleate: 0.651%<br>Metformin Hydrochloride: 0.358%<br>**Accuracy:**<br>Glibenclamide: 97.453%<br>Rosiglitazone maleate: 99.343%<br>Metformin Hydrochloride: 97.446%<br>**Limit of Detection:**<br>Glibenclamide: 80 ng/band<br>Rosiglitazone maleate: 80ng/band<br>Metformin Hydrochloride:48ng/band<br>**Limit of Quantification:**<br>Glibenclamide: 200ng/band<br>Rosiglitazone maleate: 200ng/band<br>Metformin Hydrochloride: 120ng/band<br>**Robustness:**<br>Glibenclamide: 1.218<br>Rosiglitazone maleate: 1.356<br>Metformin Hydrochloride: 0.973 | 51 |
| Empagliflozin | **Stationary phase:**Aluminium plates precoated with silica gel 60 F254 Plates (10 x 10)<br>**Mobile phase**: Toluene: Methanol (7:3 v/v)<br>**Wavelength**: 228 nm | **Linearity:** 0.5-5.0 µg/band<br>**Precision:**<br>**Inter-day precision:**0.4 %<br>**Intra-day precision:** 0.5 %<br>**Accuracy:**98.88%- 100.26%<br>**Limit of Detection:** 0.171 µg/band<br>**Limit of Quantification:** 0.521 µg/band<br>**Robustness:** Robust | 52 |

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|---|---|---|---|
| Vildagliptin And Metformin Hydrochloride | **Stationary phase:** Silica gel precoated aluminium plate 60 F254, [(20 × 10 cm) with 250 µm thickness<br>**Mobile phase**: Hexane: Methanol: Acetonitrile: Glacial acetic acid (2:3.5:2.5:0.2 v/v/v/v)<br>**Wavelength**: 217 nm | **Linearity:**<br>Vildagliptin: 10-150 ng/band<br>Metformin Hydrochloride: 50-500 ng/band<br>**Precision:**<br>Vildagliptin: 1.7%<br>Metformin Hydrochloride: 1.5%<br>**Accuracy:**<br>Vildagliptin: 99.08–103.60 %<br>Metformin Hydrochloride:98.84-102.50 %<br>**Limit of Detection:**<br>Vildagliptin: 1.74 ng/band<br>Metformin Hydrochloride: 8.2 ng/band<br>**Limit of Quantification:**<br>Vildagliptin: 5.74 ng/band<br>Metformin Hydrochloride: 27.06 ng/band<br>**Robustness:**Robust<br>**Selectivity:** selective<br>**Specificity:**specific | 53 |
| Glicazide and Metformin Hydrochloride | **Stationary phase:**pre coated with silica gel 60F254 (20*20cm).<br>**Mobile phase**: Toluene: Acetonitrile: Ethanol: Ammonium sulphate (0.25%) (4:4:4:3 v/v/v).<br>**Wavelength**: 228 nm. | **Linearity:**<br>Glicazide:200-1000 ng/spot<br>Metformin Hydrochloride:200-1000 ng/spot<br>**Precision**<br>**Intra-day Precision:**<br>Glicazide:0.24%<br>Metformin Hydrochloride: 0.29%<br>**Inter-day Precision:**<br>Glicazide:0.29%<br>Metformin Hydrochloride:0.31%<br>**Accuracy:**<br>Glicazide:98.29%<br>Metformin Hydrochloride:98.5%<br>**Limit of Detection:**<br>Glicazide: 86.14 ng/spot<br>Metformin Hydrochloride:106.11 ng/spot<br>**Limit of Quantification:**<br>Glicazide:261.03 ng/spot<br>Metformin Hydrochloride:321.54 ng/spot | 54 |
| Alogliptin benzoate and Pioglitazone hydrochloride | **Stationary phase:** Pre-coated silica gel 60F254 HPTLC plates (20 × 10 cm).<br>**Mobile phase**: Acetonitrile: 1% ammonium acetate in Methanol (4.5:5.5 v/v). | **Linearity:**<br>Alogliptin benzoate:500-3000 ng/band<br>Pioglitazone hydrochloride: 500-3000 ng/band<br>**Precision**<br>**Intra-day Precision:** | 55 |

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|---|---|---|---|
| | **Wavelength**: 254 nm. | Alogliptin benzoate: 0.76%<br>Pioglitazone hydrochloride:0.95%<br>**Inter-day Precision:**<br>Alogliptin benzoate: 0.40%<br>Pioglitazone hydrochloride:0.51%<br>**Accuracy:**<br>Alogliptin benzoate:98.96%<br>Pioglitazone hydrochloride:99.75%<br>**Limit of Detection:**<br>Alogliptin benzoate: 0.30 ng/band<br>Pioglitazone hydrochloride: 0.73 ng/band<br>**Limit of Quantification:**<br>Alogliptin benzoate: 0.91 ng/band<br>Pioglitazone hydrochloride: 2.23 ng/band<br>**Specificity:** Specific<br>**Robustness:** Robust | |
| Teneligliptin hydrobromide hydrate | **Stationary phase:** pre coated with 250 µm layer of silica gel 60 F254 (10 x 10cm)<br>**Mobile phase:** butanol: water: glacial acetic acid (6:2:2 v/v/v).<br>**Wavelength:** 245 nm. | **Linearity:** 250-1250 ng/band<br>**Precision**<br>**Intra-day Precision:** 0.65- 1.72 %<br>**Inter-day Precision:** 0.78- 1.84%<br>**Accuracy:** 98.58- 99.24%<br>**Limit of Detection:** 60.50 ng/band<br>**Limit of Quantification:** 183.36 ng/band<br>**Specificity:** Specific | 56 |
| Sitagliptin and Metformin | **Stationary phase:**precoated silica gel 60 F254 (0.2 mm layer thickness) (10 cm × 10 cm)<br>**Mobile phase**: BuOH-CHCl3 - MeOH-(CH3CH2 )2NH2 - HCOOH (4:0.5:2.5:1.5:0.06 v/v**)**<br>**Wavelength**: 206 nm. | **Linearity:**<br>Sitagliptin: 2-7µg/band<br>Metformin: 20-70 µg/band<br>**Precision :**<br>**Intra-day precision:**<br>Sitagliptin: 0.2981%<br>Metformin: 0.3780%<br>**Inter-day precision:**<br>Sitagliptin: 0.3245%<br>Metformin: 0.3154%<br>**Accuracy:**<br>Sitagliptin: 98.99 %<br>Metformin:99.05%<br>**Limit of Detection:**<br>Sitagliptin: 0.014 µg<br>Metformin: 0.013 µg<br>**Limit of Quantification:**<br>Sitagliptin: 0.042 µg<br>Metformin: 0.040 µg<br>Robustness: Robust | 57 |
| rosiglitazone maleate and metformin | **Stationary Phase:** aluminium plates with 250 mm thickness | **Linearity:**<br>Rosiglitazone Maleate: 0.4–2.0 mg band- | 58 |

| | | | |
|---|---|---|---|
| hydrochloride | precoated with silica gel 60 (F254) (20*10cm)<br>**Mobile phase**: Methanol: Water:NH4Cl 1% w/v (5:4:1 v/v/v)<br>**Wavelength**: 230 nm. | [1]<br>Metformin Hydrochloride: 20.0–100.0 mg band$^{-1}$<br>**Accuracy:**<br>Rosiglitazone Maleate: 0.88- 1.21 %<br>Metformin Hydrochloride: 0.87- 0.91%<br>**Limit of Detection:**<br>Rosiglitazone Maleate: 0.13 µg/ml<br>Metformin Hydrochloride: 6.67 µg/ml<br>**Limit of Quantification:**<br>Rosiglitazone Maleate: 0.40 µg/ml<br>Metformin Hydrochloride: 20.00 µg/ml<br>**Robustness:** Robust<br>**Selectivity:** Selective<br>**Specificity:** Specific | |
| Linagliptin and metformin | **Stationary Phase:**pre-coated silica gel 60 F254 (0.2 mm layer thickness) (10 cm × 10 cm)<br>**Mobile phase**: acetone: methanol: chloroform: formic acid (3:1:5:1v/v)<br>**Wavelength**: 230 nm | **Precision:**<br>Linagliptin:<br>0.29- 0.70%<br>Metformin:<br>0.83 - 1.48 %<br>**Accuracy:**<br>Linagliptin: 98-102 %.<br>Metformin: 98-102 %.<br>**Limit of Detection:**<br>Linagliptin: 5.19 ng/spot<br>Metformin: 8.72 ng/ spot<br>**Limit of Quantitation:**<br>Linagliptin:15.74 ng/spot<br>Metformin: 26.44 ng/spot<br>**Specificity:** Specific<br>**Robustness:** Robust | 59 |
| Metformin Hydrochloride And Glibenclamide | **Stationary Phase:** Precoated silica Gel 60 F254 aluminium sheet<br>**Mobile phase**: Methanol: Water: 0.4 % Sodium sulphate (7:5:11).<br>**Wavelength**: Metformin hydrochloride and Glibenclamide was performed at 232 nm and at 238 nm respectively. | **Linearity:**<br>Metformin Hydrochloride: 250 ng to 1750 ng/spot<br>Glibenclamide: 250 ng to 1750 ng/spot<br>**Precision:**<br>**Intra- day precision:**<br>Metformin Hydrochloride: 1.90%<br>Glibenclamide: 0.92 %<br>**Inter-day precision:**<br>Metformin Hydrochloride: 1.52%<br>Glibenclamide: 1.67%<br>**Accuracy:**<br>Metformin Hydrochloride: 95.79 %-97.84 %<br>Glibenclamide: 96.0 % - 97.03 %<br>**Limit of Detection:**<br>Metformin Hydrochloride: 1.2412 mcg<br>Glibenclamide:0.9944 mcg | 60 |

| | | | |
|---|---|---|---|
| | | **Limit of Quantitation:**<br>Metformin Hydrochloride: 3.7613 mcg<br>Glibenclamide:3.0133 mcg<br>**Specificity:** specific<br>**Robustness:** Robust | |
| Metformin Hydrochloride And Sitagliptin Phosphate | **Stationary Phase:**precoated silica gel 60 F254 TLC plates (10 × 10 cm)<br>**Mobile phase**: Butanol: Water: Glacial acetic acid (6:2: 2, v/v/v)<br>**Wavelength**: 227 nm | **Linearity:**<br>Metformin Hydrochloride: 500-10000 ng/band<br>Sitagliptin Phosphate: 50-1000 ng/band<br>**Precision:**<br>**Intra- day precision:**<br>Metformin Hydrochloride:0.49<br>Sitagliptin Phosphate:0.72<br>**Inter-day precision:**<br>Metformin Hydrochloride: 1.13<br>Sitagliptin Phosphate: 1.43<br>**Accuracy:**<br>Metformin Hydrochloride: 98.14-101.64%<br>Sitagliptin Phosphate:99.29- 101.06%<br>**Limit of Detection:**<br>Metformin Hydrochloride: 13.05 ng/µL<br>Sitagliptin Phosphate: 2.65 ng/µL<br>**Limit of Quantitation:**<br>Metformin Hydrochloride: 39.56 ng/µL<br>Sitagliptin Phosphate: 8.03 ng/µL<br>**Selectivity :** Selective | 61 |
| Pioglitazone Hcl and Telmesarton Hcl | **Stationary Phase:**precoated silica gel 60 F254 TLC plates<br>**Mobile phase** Toluene: Ethyl acetate: Methanol (7: 2: 1 v/v/v)<br>**Wavelength**: 269 nm | **Accuracy:**<br>Pioglitazone Hcl: 100.12<br>Telmesarton Hcl: 99.08<br>**Precision:**<br>Pioglitazone Hcl: 0.56<br>Telmesarton Hcl: 1867.51<br>**Limit of Detection:**<br>Pioglitazone Hcl: 140 ng/spot<br>Telmesarton Hcl:188 ng/spot<br>**Limit of Quantitation:**<br>Pioglitazone Hcl:140.06 ng/spot<br>Telmesarton Hcl: 186.75 ng/spot<br>**Robustness:**Robust | 62 |
| Remogliflozin etabonate | **Stationary Phase:**Precoated silica gel F254 TLC plates (10*10cm)<br>**Mobile phase**methanol: ethyl acetate:<br>toluene: NH3 (2:4:4:0.1, v/v/v)<br>**Wavelength**: 229 nm | **Linearity:**500-8000 ng/band<br>**Accuracy:** 97.73- 99.19%<br>**Precision:**<br>**Intra- day precision:** 0.49-1.84 %<br>**Inter-day precision:** 1.13- 1.99%<br>**Limit of Detection:** 100 ng/band<br>**Limit of Quantitation:** 300 ng/band<br>**Specificity:** Specific<br>**Robustness:**Robust | 63 |

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**RESEARCH ARTICLE**

# Analyzing Agricultural Data using Inverse Lindley Distribution with Fuzzy Parameters

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**ABSTRACT**

In many practical circumstances, we are presented with data that is not just random but also imprecise. To address these uncertainties, fuzzy concepts should be integrated into statistical techniques. This work examines how to estimate the Inverse Lindley distribution with fuzzy parameters and investigated maximum likelihood and Bayesian methods using fuzzy data. This article focuses on estimating parameters for the inverse Lindley distribution with fuzzy observations. The unknown model parameter was estimated using both classical and Bayesian approaches. The classical methodology involves estimating population parameters using the maximum likelihood (ML) and maximum product of distances (MPS) methods. Bayesian estimate relies on the squared error loss function (SELF) and the Markov Chain Monte Carlo (MCMC) approach. The asymptotic confidence intervals and highest posterior density (HPD) credible intervals for the unknown parameter are calculated. The estimators' performances are compared using their mean squared errors. Finally, a real time agriculture data set of Puducherry in year of 2019 is analyzed to provide plots examples of the PDF, CDF, Hazard Rate Function, and Survival Function is discussed. As a result of the comparison, fuzziness values were found to be more precisely calculated than real-time data.

**Keywords:** inverse Lindley distribution, maximum likelihood (ML), PDF, CDF, MLE.
**Mathematical Subject Classification (2020):** 03E72, 62B15.

## INTRODUCTION

In statistics, estimate is the process of making judgments about a population. These conclusions are based on data from the same population. There are numerous estimation methods accessible in the literature, each with merits and

limitations. Some well-known estimating methods are the method of maximum likelihood, method of least square, method of moments, etc. Zadeh created the phrase "fuzzy variable" in 1965 [1] to indicate inappropriate linguistic idioms and vernacular. This was the beginning of fuzzy set theory. A fuzzy set is made up of components with various membership levels. This author [2] discusses the many approaches for estimating the probability density function (PDF) and cumulative distribution function (CDF) for the Lindley Distribution. The following estimation methods are considered: uniformly minimum variance unbiased estimator (UMVUE), maximum likelihood estimator (MLE), percentile estimator (PCE), least square estimator (LSE), weighted least square estimator (WLSE), Cramér-von-Mises estimator (CVME), and Anderson-Darling estimator (ADE). Monte Carlo simulations are used to evaluate the performance of the proposed estimate methods. The authors [3], investigated various approaches of estimating the unknown parameters of GILD. First, we briefly describe different estimation methods, such as maximum likelihood estimators, moments estimators, least squares estimators, weighted least squares, maximum product spacing estimates, minimum distance methods, Cramer-von-Misses methods, and Anderson-Darling methods, and compare them using extensive numerical simulations.

The task of estimating the parameters of the generalized Lindley distribution [4], In addition to the classical estimator, inverse moment and modified inverse estimators are presented and their properties explored. A criterion for the existence and uniqueness of the inverse moment and modified inverse estimators of the parameters is defined. Monte Carlo simulations are used to compare the estimators' performance. Two methods for building joint confidence zones for the two parameters are also shown, and their performance is discussed. A real-world example is provided to demonstrate the recommended methods. Estimate the probability density function and cumulative density function of the inverse Weibull distribution. The following estimators are examined. Maximum likelihood estimator uniformly minimum variance unbiased estimator [5], the bias and mean squared error are expressed analytically. Simulation experiments and a real-world application demonstrate that the maximum likelihood estimator works better. The EM algorithm is used to calculate the parameter's maximum likelihood estimate (MLE) and provide an approximate confidence interval based on the MLE's asymptotic normalcy. In the Bayesian context, we construct the parameter's Bayes estimate using a Laplace approximation and a Markov Chain Monte Carlo approach. In addition, the maximum posterior density credible interval for the unknown parameter is determined. Extensive simulations are used to compare the performance of the various proposed approaches [6].

Because the Lindley distribution is more flexible than the exponential distribution, the same estimators have been developed and compared [7]. Monte Carlo simulations and real-world data analysis are used to compare the performance of the proposed estimation methods. The purpose of this work [8] is to estimate the unknown parameters of the inverse Weibull (IW) distribution using eight different estimation methods: maximum likelihood (ML), least squares (LS), weighted least squares (WLS), percentile (PC), maximum product of spacing (MPS), probability weighted moments (PWM), Cramér-von Mises (CM), and Anderson-Darling. The performance of different estimating approaches is compared using a large Monte Carlo simulation. Their robustness properties are also examined. At the conclusion of the study, two real data sets are examined for illustration and comparison.

Bayes estimators were used with gamma priors on both the shape and scale parameters of the generalised Lindley distribution, resulting in the largest posterior density interval estimations. Bayesian estimation is investigated with regard to both symmetric (squared error) and asymmetric (linear-exponential (LINEX)) loss functions. Finally [9], generate Bayesian prediction estimates and predictive interval estimates for future record values. To demonstrate the findings, a real data set is analyzed, and Monte Carlo simulations are run to assess the performance of the suggested estimate and prediction techniques. The maximum likelihood, uniformly minimal variance unbiased, and Bayes estimators are discussed here. The exact confidence intervals for the probability density and cumulative distribution functions of the inverse Lindley distribution are also calculated. Real-world data applications and simulation studies are used to demonstrate and compare the methodologies developed thus far [10]. Then explore the subject of classical and Bayesian estimate of the inverse Lindley distribution [11] parameter based on lower records, as well as predicting future record values. We get the (ML) estimator, the approximate confidence interval, and two boot-strap types for the parameter from ILD records. In Bayesian estimation, employ Tierney Kadane and MCMC approaches.

Future record values were also investigated using ML and Bayesian techniques. The highest conditional density and credible intervals are created for a future lower record. The current work used real-time data to investigate the probability density function, cumulative density function, Hazard rate function, and Survival function of the Inverse Lindley distribution with fuzzy parameters. This study focused on Puducherry agriculture in the year of 2019 [12].

**Fuzzy Mathematical tools**

**Inverse Lindley Distribution with fuzzy Parameters**

The distribution is unique in that it contains a single parameter, similar to the exponential distribution, but instead of a constant hazard rate, it has a unique modal, non-monotone, up-side-down bathtub (UBT) shape. The probability density function (PDF) and cumulative distribution function (CDF) of ILD are provided as:

$$f(x;\theta) = \frac{\theta^2}{(1+\theta)}\left(\frac{1+x}{x^3}\right)e^{-\frac{\theta}{x}}; x > 0, \theta > 0.$$

$$F(x;\theta) = \left[\left(1 + \frac{1+x}{(1+\theta)x}\right)\right]e^{-\frac{\theta}{x}}; x > 0, \theta > 0.$$

$\theta$ Represents the scaling parameter, ILD ($\theta$) has closed-form formulas for reliability and hazard rate, making it ideal for modeling lifespan data.

$$R(x;\theta) = 1 - \left[\left(1 + \frac{1+x}{(1+\theta)x}\right)\right]e^{-\frac{\theta}{x}}; x > 0, \theta > 0.$$

$$H(x;\theta) = \frac{\theta^2(1+x)}{x^2[x(1+\theta)\left(e^{\frac{\theta}{x}} - 1\right) - \theta]}; x > 0, \theta > 0.$$

**Maximum likelihood estimate**

Let $X_1, X_2, \dots, X_n$ be a sequence of independent observations on a random variable X. The CDF and PDF are given in Equations respectively. The likelihood equation for observing accurate realizations of $X_1, X_2, \dots, X_n$ is as follows:

$$l(x;\theta) = \left(\frac{\theta^2}{1+\theta}\right)^n e^{-\theta\sum_{i=1}^{n}\frac{1}{x_i}}\prod_{i=1}^{n}\left(\frac{1+x_i}{x_i^3}\right); x, \theta > 0$$

However, in this case, we assume that we have just a limited understanding of the variable's observed values. Because they are not in precise form and are given with some mistake, the investigator can only provide estimated lifespan of the objects as guess values by indicating a tiny interval around the observed value. Furthermore, the investigator forms this interval based on his beliefs about the interval's different values. Such uncertainty in observed data can be properly modeled using the membership function of a fuzzy number. As a result, we can infer that the observed data is fuzzy, with the inherent unpredictability integrated into the possibility distribution. Let $\tilde{x}_1, \tilde{x}_2, \dots, \tilde{x}_n$ _n denote the observed fuzzy lifetimes where $\tilde{x}_i = (a_i, b_i, c_i); i = 1,2, \dots, n$, with the associated membership functions $\xi_{\tilde{x}_1}(), . \xi_{\tilde{x}_2}(), . \dots, \xi_{\tilde{x}_n}(.)$ such that

$$\xi_{\tilde{x}_i}(x) = \begin{cases} \frac{x - (x_i - h)}{h}; & x_i - h \le x \le x_i \\ \frac{(x_i + h) - x}{h}; & x_i \le x \le x_i + h \\ 0; & \text{otherwise} \end{cases}$$

Where h can be chosen appropriately based on the past knowledge we possess. Naturally, the value of the component h will differ from problem to problem depending on its nature and the degree of error in the observed data. The joint membership function of the observed fuzzy data vector $\tilde{x} = \{\tilde{x}_1, \tilde{x}_2, \dots, \tilde{x}_n\}$ can be expressed as:

$$\xi_{\tilde{x}}(x) = \prod_{i=1}^{n}\xi_{\tilde{x}_i}(x)$$

According to Zadeh's (1968) [1] definition of probability for a fuzzy event, the likelihood function of the observed fuzzy data is stated as:

$$l(\theta;\tilde{x}) = P(\tilde{x};\theta) = \int f(x;\theta)\xi_{\tilde{x}}(x)dx$$

Substituting the values of $f(x;\theta)$ and $\xi_{\tilde{x}}(x)$ from above yields the likelihood equation:

$$l(\theta;\tilde{x}) = \prod_{i=1}^{n} \int f(x;\theta)\xi_{\tilde{x}_i}(x)dx$$
$$= \prod_{i=1}^{n} \int \left(\frac{\theta^2}{1+\theta}\right)\left(\frac{1+x}{x^3}\right) e^{-\frac{\theta}{x}}\xi_{\tilde{x}}(x)dx$$

After taking the logarithm of the likelihood function, we obtain

$$L(\theta;\tilde{x}) = \log L(\theta;\tilde{x}) = 2n\log\theta - n\log(1+\theta) + \sum_{i=1}^{n} \log\int \left(\frac{1+x}{x^3}\right) e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx$$

To obtain the MLE of θ, we will maximize the log-likelihood equation. We differentiate the previous equation with regard to parameter θ and set the derivative to zero.

$$\frac{\partial \ln G(\theta;\tilde{x}_i)}{\partial\theta} = \frac{2n}{\theta} - \frac{n}{(1+\theta)} - \sum_{i=1}^{n} \frac{\int \left(\frac{1+x}{x^4}\right) e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx}{\int \left(\frac{1+x}{x^3}\right) e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx} = 0$$

Solving the equation for θ yields the estimated value of the parameter. However, it is clear that the given equation does not return an explicit answer. Iterative numerical methods could be an appropriate way for estimating the parameters of such equation. We propose the Newton-Raphson technique to estimate the parameter based on an appropriate starting guess.

**Maximum product of spacing**

The concept of the product of spacing is quite close to that of maximum likelihood. To construct the likelihood equation, we write the sample observations' joint density. Similarly, we can calculate the product of spacing by calculating the geometric mean of the spacing between ordered sample observations. Let $f(x)$ and $F(x)$ be the PDF and CDF of the distribution, respectively. The density of f(x) is strictly positive in the interval $(m,n)$ and zero everywhere else, i.e. $m < x_{(1)} < x_{(2)} < \cdots < x_{(n)} < n$.. Here, we assume that X is a continuous variable with support (0,∞), resulting in $m = 0$ and $n = \infty$. As a result, the border points of PDF and CDF will be as follows:

$$F(x) = 0 = f(x)\forall x < m \text{and } F(x) = 1; f(x) = 0\forall x > n$$

Let $x_{i:n}$ represent the i^th order statistics. The ith spacing $D_i$ is defined as follows:

$$D_i = F(x_{i:n}) - F(x_{i-1:n}); i = 1,2,\ldots,(n+1)$$

We must obtain the CDF value utilizing imprecise or fuzzy observations. This ambiguity in the observed data is addressed with fuzzy numbers by creating a triangle membership function for each observation based on prior knowledge of the nature of imprecision. Thus, CDF$F(\theta;\tilde{x})$ corresponding to an observed fuzzy numbers $\tilde{x} = (a,b,c)$, can be expressed as:

$$F(\theta;\tilde{x}) = P(X \le a) + \int P(a \le X \le x)\xi_{\tilde{x}_i}(x)dx$$
$$= F(a) + \int \big(F(x) - F(a)\big)\xi_{\tilde{x}}(x)dx$$

$$F(\theta;\tilde{x}) = \left[1+\frac{\theta}{(1+\theta)a}\right]e^{-\frac{\theta}{a}} + \int\left[\left(1+\frac{\theta}{(1+\theta)x}\right)e^{-\frac{\theta}{x}} - \left(1+\frac{\theta}{(1+\theta)a}\right)e^{-\frac{\theta}{a}}\right]\xi_{\tilde{x}_i}(x)dx$$

$$G(\theta;\tilde{x}) = \left[\prod_{i=1}^{n+1} D_i\right]^{(1/n+1)}$$

$$S(\theta;\tilde{x}) = \log G(\theta;\tilde{x}) = \frac{1}{n+1}\sum_{i=1}^{n+1}\log D_i$$

$$= \frac{1}{n+1}\sum_{i=1}^{n+1}\log[F(\theta;\tilde{x}_i) - F(\theta;\tilde{x}_{i-1})]$$

where,

$$F(\theta;\tilde{x}_i) - F(\theta;\tilde{x}_{i-1}) = \left[F(a_i) + \int\left(F(x_i) - F(a_i)\right)\xi_{\tilde{x}_i}(x)dx\right] - \left[F(a_{i-1}) + \int\left(F(x_{i-1}) - F(a_{i-1})\right)\xi_{\tilde{x}_{i-1}}(x)dx\right]$$

$$\frac{\partial \ln G(\theta;\tilde{x}_i)}{\partial\theta} = \sum_{i=1}^{n+1}\frac{1}{F(\theta;\tilde{x}_i) - F(\theta;\tilde{x}_{i-1})}\left[\left\{\omega(\theta,a_i) + \int\{\omega(\theta,x_i) - \omega(\theta,a_i)\}\xi_{\tilde{x}_i}(x)dx\right\} - \left\{\omega(\theta,a_{i-1}) + \int\{\omega(\theta,x_{i-1}) - \omega(\theta,a_{i-1})\}\xi_{\tilde{x}_{i-1}}(x)dx\right\}\right]$$

where,

$$\omega(\theta,x) = \frac{\partial}{\partial\theta}\left(\left\{1+\frac{\theta}{(1+\theta)x}\right\}e^{-\theta/x}\right) = -\frac{e^{-\theta/x}[\theta^2(x+1)+\theta(2x+1)]}{x^2(1+\theta)^2}$$

Solving the aforementioned non-linear equation in θ yields the MPS estimate of the population parameter θ. Furthermore, it is clear that no closed solution to the preceding equation exists. Therefore, we shall apply numerical approaches to acquire the solution.

**Asymptotic confidence interval**

The distribution of MLE and MPS estimators, $\hat{\theta}_{ML}$ and $\hat{\theta}_{MPS}$, is not explicitly known, making it difficult to obtain accurate interval estimates.

$$\left(\hat{\theta}_{MPS} - \theta\right) \xrightarrow{D} N(0, I^{-1}(\theta))$$

Using MLE and MPS estimations, the estimated 100(1-$\alpha$)% asymptotic confidence interval for parameter θ is as follows:

$$CI_{ML} = \left[\hat{\theta}_{ML} \pm z_{\alpha/2}\sqrt{I^{-1}\left(\hat{\theta}_{ML}\right)}\right]$$
$$CI_{MPS} = \left[\hat{\theta}_{MPS} \pm z_{\alpha/2}\sqrt{I^{-1}\left(\hat{\theta}_{MPS}\right)}\right]$$

The observed Fisher information $I\left(\hat{\theta}_{ML}\right)$ is defined as:

$$I(\hat{\theta}_{ML}) = -\left(\frac{\partial^2 L(\theta;\tilde{x})}{\partial\theta^2}\right)_{\theta=\hat{\theta}_{ML}}$$

$$\frac{\partial^2 L(\theta;\tilde{x})}{\partial\theta^2} = \frac{-2n}{\theta^2} + \frac{n}{(1+\theta)^2} + \sum_{i=1}^{n}\left[\frac{\int\left(\frac{1+x}{x^5}\right)e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx}{\int\left(\frac{1+x}{x^3}\right)e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx} - \left(\frac{\int\left(\frac{1+x}{x^4}\right)e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx}{\int\left(\frac{1+x}{x^3}\right)e^{-\frac{\theta}{x}}\xi_{\tilde{x}_i}(x)dx}\right)^2\right]$$

Similarly, we can get the observed Fisher information $I(\hat{\theta}_{MPS})$ as follows:

$$I(\hat{\theta}_{MPS}) = -\left(\frac{\partial^2 S(\theta;\tilde{x})}{\partial\theta^2}\right)_{\theta=\hat{\theta}_{MPS}}$$

Let

$$N(\theta,x) = [\{\omega(\theta,a_i) + \int\{\omega(\theta,x_i) - \omega(\theta,a_i)\}\xi_{\tilde{x}_i}(x)dx\} - \left\{\omega(\theta,a_{i-1}) + \int\{\omega(\theta,x_{i-1}) - \omega(\theta,a_{i-1})\}\xi_{\tilde{x}_{i-1}}(x)dx\right\}]$$

The second-order partial derivative of the logarithm of product spacing function S is provided as follows:

$$\frac{\partial^2 S(\theta;\tilde{x})}{\partial\theta^2} = \sum_{i=1}^{n+1}\left[\frac{N'(\theta,x)}{F(\theta;\tilde{x}_i) - F(\theta;\tilde{x}_{i-1})} - \left(\frac{N(\theta,x)}{F(\theta;\tilde{x}_i) - F(\theta;\tilde{x}_{i-1})}\right)^2\right]$$

where

$$N'(\theta,x) = \frac{\partial N(\theta,x)}{\partial\theta} = \left[\left\{\omega'(\theta,a_i) + \int\{\omega'(\theta,x_i) - \omega'(\theta,a_i)\}\xi_{\tilde{x}_i}(x)dx\right\} - \left\{\omega'(\theta,a_{i-1}) + \int\{\omega'(\theta,x_{i-1}) - \omega'(\theta,a_{i-1})\}\xi_{\tilde{x}_{i-1}}(x)dx\right\}\right]$$

and

$$\omega'(\theta,x) = \frac{\partial\omega(\theta,x)}{\partial\theta} = \frac{e^{-\theta/x}[\theta(x+1)\{\theta(1+\theta) - 2x\} + (2x+1)\{\theta(1+\theta) - x(1-\theta)\}]}{x^3(1+\theta)^3}$$

**Estimation of reliability and hazard function**

By creating a mechanism for getting MLE and MPS from fuzzy data, we can easily estimate reliability and hazard function by inserting the estimated parameter value into their functional forms.

$$R(t;\hat{\theta}) = 1 - \left[1 + \frac{\hat{\theta}}{(1+\hat{\theta})t}\right]e^{-\frac{\hat{\theta}}{t}}$$

$$H(t;\hat{\theta}) = \frac{\hat{\theta}^2(1+t)}{t^2\left[t(1+\hat{\theta})\left(e^{\frac{\hat{\theta}}{t}} - 1\right) - \hat{\theta}\right]}$$

The parameter estimate ($\theta$i.e.$\hat{\theta}_{ML}$ or $\hat{\theta}_{MPS}$) is denoted as $\hat{\theta}$ . Substituting the estimated values of parameters $\hat{\theta}_{ML}$L and $\hat{\theta}_{MPS}$ in the preceding formulas yields the estimated reliability and hazard function at every time point t corresponding to the estimated value of θ.

**Bayesian estimation**

This section covers the Bayesian estimation of population parameter θ using fuzzy data. In Bayesian analysis, the parameter θ is considered a random variable. To model its change, we must provide a distribution known as the population parameter's prior distribution. Using the Bayes theorem, we update the prior information about the unknown parameter θ based on the likelihood of the sample observations. The gamma prior is chosen since it is versatile and has the same support as the parameter θ (0,∞). The probability density function of the prior with hyper parameters p and q is given below:

$$\pi(\theta) = \frac{p^q}{\Gamma(q)} e^{-p\theta}\, \theta^{(q-1)}; \theta > 0, q > 0, p > 0$$

Using the prior in above equation one can easily calculate the posterior distribution of the parameter θ for the supplied data.

$$\pi(\theta \mid \tilde{x}) = \frac{l(\theta; \tilde{x})\pi(\theta)}{\int_0^\infty l(\theta; \tilde{x})\pi(\theta) d\theta}$$

where,

$$J = e^{-p\theta}\, \theta^{2n+q-1}(1+\theta)^{-n} \prod_{i=1}^{n} \int \left(\frac{1+x}{x^3}\right) e^{-\frac{\theta}{x}} \xi_{\tilde{x}_i}(x) dx$$

To estimate the parameter under the Bayesian paradigm, we used the quadratic loss function, which is defined as follows:

$$l_S(\hat{\theta}, \theta) = \epsilon(\hat{\theta} - \theta)^2$$

The estimated value of parameter θ is denoted as $\hat{\theta}$, and ϵ can be a function of θ. If ϵ equals 1, we have SELF. This is the most popular symmetric loss function. It treats both overestimation and underestimate of parameters equally. Furthermore, it penalizes huge errors significantly more than tiny errors. If h(.) is a function of θ, then the Bayes estimate of h(.) under SELF equals the expectation of the posterior distribution.

$$\hat{h}(\theta) = E_\pi[h(\theta)]$$

This approach generates sample values of parameter θ using a proposal distribution with stationary distribution π(θ). The main steps of the algorithm are listed below:

Step 1: Begin with an initial guess value $\theta^0$ and set $j = 1$.
Step 2: Create a new candidate parameter value θ^* at the jth step using the proposal density $q\left(\theta^{(j)} \mid \theta^{(j-1)}\right)$
Step 3: Accept candidate $\theta^*$ as

$$\theta^{(j)} = \begin{cases} \theta^* & \text{with probability} \quad \rho\left(\theta^*, \theta^{(j-1)}\right) \\ \theta^{(j-1)} & \text{with probability} \quad 1 - \rho\left(\theta^*, \theta^{(j-1)}\right) \end{cases}$$

where

$$\rho\left(\theta^*, \theta^{(j-1)}\right) = \min\left\{\frac{\pi(\theta^* \mid \hat{u}) q\left(\theta^{(j-1)} \mid \theta^*\right)}{\pi(\theta^{(j-1)} \mid \hat{u}) q(\theta^* \mid \theta^{(j-1)})}, 1\right\}$$

Step 4: Calculate $R^{(j)}(t) = 1 - \left[1 + \frac{\theta^{(j)}}{(1+\theta^{(j)})x}\right] e^{-\frac{\theta^{(j)}}{x}}$ and $H^{(j)}(t) = \frac{\theta^{(j)^2}(1+x)}{x^2\left[x(1+\theta^{(j)})\left(e^{\frac{\theta^{(j)}}{x}} - 1\right) - \theta^{(j)}\right]}$

Step 5: Repeat steps 2-4 M times to produce $\theta^{(j)}, R^{(j)}(t)$ and $H^{(j)}(t); j = 1,2,\dots,M$ where j=1,2,…,M Bayes estimates for parameters θ, R(t), and H(t) under SELF are as follows:

$$\hat{\theta}_B = E_\pi(\theta \mid \tilde{u}) = \frac{1}{M - M_0} \sum_{j=M_0+1}^{M} \theta^{(j)}$$

$$\hat{R}(t)_B = E_\pi(R(t) \mid \tilde{u}) = \frac{1}{M - M_0} \sum_{j=M_0+1}^{M} R^{(j)}(t)$$

$$\hat{H}(t)_B = E_\pi(H(t) \mid \tilde{u}) = \frac{1}{M - M_0} \sum_{j=M_0+1}^{M} H^{(j)}(t)$$

Where $M_0$ represents the burn-in period, To create the HPD credible intervals for θ, arrange the simulated samples as $\theta^{(1)} \le \theta^{(2)} \le \cdots \le \theta^{(M)}$. Create 100(1-$\alpha$)% credible intervals for θ as follows:

$$\left(\theta^{[1]}, \theta^{[M(1-\alpha)+1]}\right), \dots, \left(\theta^{[M\alpha]}, \theta^{[M]}\right)$$

Here, [X] represents the greatest integer less than or equal to X. The HPD credible interval for θ is the shortest in length.

**Real Data Analysis**

This part will evaluate the performance of developed estimators on an actual data set. In Puducherry agriculture in the year of 2019, all the seasons were given and their survival durations were recorded. Kaggle.com [12] reported this data set. The crop production data is shown in Table.1. Bayesian estimation of Inverse Lindley distribution with fuzzy parameters to given data Posterior mean of $\theta$ is 0.4531, Posterior median of $\theta$ is 0.5000 and 95% credible interval for the $\theta$is 0 to 0.5378.

## RESULTS AND DISCUSSION

Fuzzy lifespan data can be utilized to determine the reliability of Lindley distribution with fuzzy parameters. in real-world circumstances with uncontrollable elements. However, fuzzy sets only have one membership degree parameter, which leads to a less precise description of the external world. When dealing with fuzzy information, the Inverse Lindley Distribution with fuzzy parameters expresses uncertainty and fuzziness more accurately. The results demonstrated that changing the true values of various kinds of parameters resulted in a significantly smaller mean square error under Bayesian estimation compared to the maximum likelihood estimator, MPS, and that the results of Trace plot MCMC and PDF of Posterior Samples of Inverse Lindley distribution with fuzzy parameters for the given data. Kaggle.com validates the current research findings [12]. We discovered results for the Probability density function (Fig.1), Cumulative density function (Fig.2), Hazard rate function (Fig.3), and Trace plot MCMC and PDF of Posterior Samples (Fig.4.a, 4.b) of Inverse Lindley distribution with fuzzy parameters to the agriculture problem of Pondicherry in 2019 and supporting MATLAB tool, which show how well the suggested technique performs in predicting crop production for all seasons.

## CONCLUSION

This article focuses on developing an MPS estimator for non-precise observed data. We used the inverse Lindley distribution, a highly effective survival model. To evaluate MPS performance with other estimators, we obtained the MLE and Bayes estimators for the fuzzy parameter. Both classical and Bayesian techniques provide point estimates for parameters, as well as estimated reliability, hazard, and confidence intervals. The current article discusses a combination of statistical methods and a fuzzy approach to Puducherry agriculture in the year of 2019. This paper's methods can help researchers and statisticians deal with imprecise agriculture data during studies. Extending the

MPS estimation for fuzzy data using a Bayesian setting would be a highly beneficial research topic. Using fuzzy models to address fuzzy data challenges can enhance our understanding of both statistical and fuzzy approaches.

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**Table 1: Agriculture data of Pondicherry in the year of 2019**

| Crop | **Season** | **Area** | **Production** | **Annual Rainfall** | **Fertilizer** | **Pesticide** | **Yield** |
|---|---|---|---|---|---|---|---|
| Arecanut | Kharif | 52 | 78 | 1317 | 8931.52 | 19.24 | 1.5 |
| Bajra | Kharif | 14 | 36 | 1317 | 2404.64 | 5.18 | 2.57 |
| Bajra | Rabi | 1 | 3 | 1317 | 171.76 | 0.37 | 3 |
| Bajra | Summer | 7 | 18 | 1317 | 1202.32 | 2.59 | 2.57 |
| Banana | Kharif | 273 | 16637 | 1317 | 46890.48 | 101.01 | 32.76667 |
| Black pepper | Kharif | 11 | 15 | 1317 | 1889.36 | 4.07 | 1.36 |
| Cashewnut | Kharif | 145 | 65 | 1317 | 24905.2 | 53.65 | 0.426667 |
| Coconut | Whole Year | 1857 | 18661000 | 1317 | 318958.3 | 687.09 | 9474.625 |
| Cotton(lint) | Kharif | 500 | 1248 | 1317 | 85880 | 185 | 2.745 |
| Dry chillies | Kharif | 2 | 26 | 1317 | 343.52 | 0.74 | 13 |
| Dry chillies | Rabi | 3 | 41 | 1317 | 515.28 | 1.11 | 13.67 |
| Dry chillies | Summer | 2 | 26 | 1317 | 343.52 | 0.74 | 13 |
| Groundnut | Kharif | 1 | 3 | 1317 | 171.76 | 0.37 | 3 |
| Groundnut | Rabi | 231 | 693 | 1317 | 39676.56 | 85.47 | 3 |
| Groundnut | Summer | 21 | 63 | 1317 | 3606.96 | 7.77 | 3 |
| Moong(Green Gram) | Rabi | 12 | 9 | 1317 | 2061.12 | 4.44 | 0.75 |
| Moong(Green Gram) | Summer | 629 | 156 | 1317 | 108037 | 232.73 | 0.606667 |
| Onion | Kharif | 2 | 10 | 1317 | 343.52 | 0.74 | 5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Onion | Summer | 1 | 5 | 1317 | 171.76 | 0.37 | 5 |
| Other Rabi pulses | Rabi | 53 | 42 | 1317 | 9103.28 | 19.61 | 0.79 |
| Other Kharif pulses | Kharif | 5 | 4 | 1317 | 858.8 | 1.85 | 0.8 |
| Other Summer Pulses | Summer | 21 | 17 | 1317 | 3606.96 | 7.77 | 0.81 |
| Ragi | Kharif | 32 | 77 | 1317 | 5496.32 | 11.84 | 2.41 |
| Ragi | Rabi | 4 | 10 | 1317 | 687.04 | 1.48 | 2.5 |
| Ragi | Summer | 23 | 55 | 1317 | 3950.48 | 8.51 | 2.39 |
| Rice | Kharif | 4323 | 15171 | 1317 | 742518.5 | 1599.51 | 3.216667 |
| Rice | Rabi | 9397 | 28114 | 1317 | 1614029 | 3476.89 | 3.883333 |

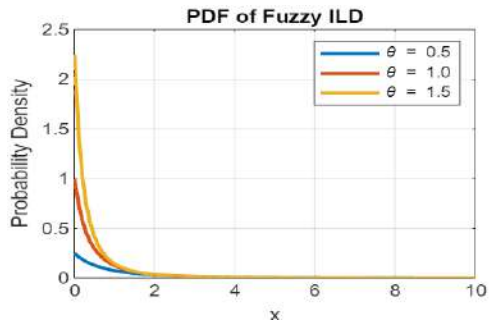


**Fig.1. Mathematical Results of the Inverse Lindley distribution with fuzzy parameters**

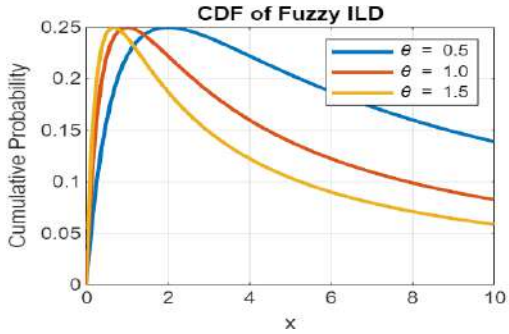


**Fig.2. : PDF of Inverse Lindley distribution with fuzzy parameters.**

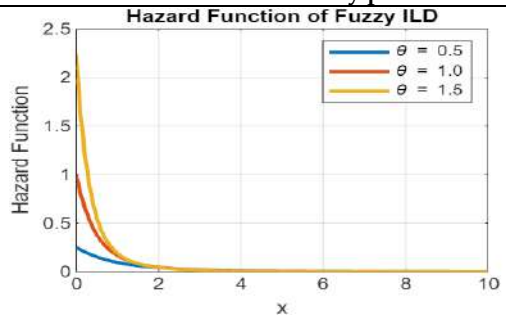


**Fig.3. Hazard rate of Inverse Lindley distribution with fuzzy parameters.**

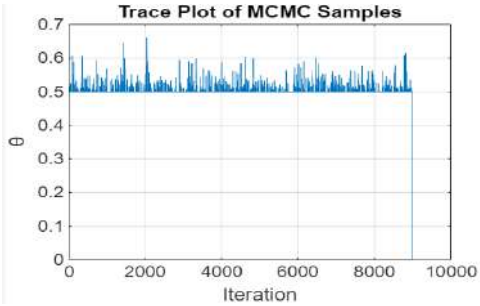


**Fig. 4. Trace plot MCMC of Inverse Lindley distribution with fuzzy parameters**

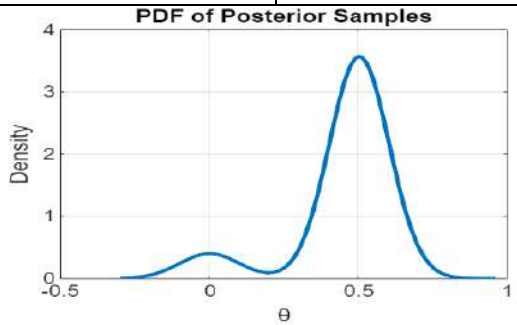


**Fig. 5 PDF of Posterior Samples of Inverse Lindley distribution with fuzzy parameters**

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**RESEARCH ARTICLE**

# The Design and Development of Surgical Nurse Assistance Robotic System by using AI with MSN Network

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## ABSTRACT

In recent trends embedded system plays major role in electronics especially in robotics. Embedded system is a combination of hardware and software which is specifically designed to perform specific task in real time. Which makes a sophisticate model design in robotic automation system will support the performance and it also promises the less operating time, higher accuracy and less percentage of risks. It involves in a different variety of application areas in consumer electronics, industrial automation, security and telecommunications, instrumentations, aerospace, automobile and bio medical health care sectors. This research is to develop a robot which can assist surgeon during surgery. Today's a lot of medical robot system is available but none can assist a doctor during a complex surgery. At the time of surgery the surgeon need the well experience nurse to handle the tools but that time also have more chance to occur errors like man made errors etc. The surgical assistance robot system has been designed for this purpose to avoid the errors and man power. It can understand the doctor's needs by them inputs and it brings the appropriate surgical tool during surgery and it also getting the details from the environmental data during the surgery. The robotic system can understand commands and it fetches the necessary tools and respectively like cotton pads and clamps to stop bleeding, fetches scissors, catheter, and needle etc.It is also monitor the patient health conditions and the environmental conditions of surgical room. These all the functions will be achieved by the MSN network and AI technologies.

**Keywords:** surgical assistance robot, MSN network and AI technologies.

## INTRODUCTION

In bio medical field the robotic automation function performs the less operating time, higher accuracy and less percentage of risks compare with the traditional methods. In this research work describe about the current trends of applications in bio medical section especially in health care sector. The various role of embedded system based robotic system to be implemented in bio medical sector like robotic automation operations in surgery. [1]The ARD is recent development method of robotic system in embedded. In recent days the Assistance Robotic Device (ARD) which is implement in bio medical section in various application. The quest of my research is to develop a robot which can assist a medical practitioner during surgery. Today, a lot of medical robots are available but none can assist a doctor during a complex surgery. Nowadays, the surgeons are in need of a surgical assistance personnel who can support them during the complex surgery. Since surgery can be ascertained based on advancement of the disease for the patient, only the experienced nurses in that field can render their support. For example, an ortho based surgery needs a surgical assistance that should be well known about the surgical tools that are used in mending bones, ligaments and cartilages.

The surgical assistance robot has been designed for this purpose. It can understand the surgeon's situation and based on inputs and bring the appropriate surgical tool during surgery. This system can also monitor the patient health condition and environmental conditions of surgery room through the multiple sensor network (MSN) module. The platform chosen to develop the robot is embedded systems. Because the embedded system is the stable and best platform to produce the closed control system with enhancing features to connect with any platform if required. The sophisticated embedded systems that can be used in robots have enormous hardware and software complexities that may need ASIPs, IPs, PLAs, scalable or configurable processors.

## LITERATURE REVIEW

❖ Embedded system in biomedical applications: challenges ahead BY PriyaPanneerselvam(2014) (IJSETR).
This paper focuses the embedded systems development especially in biomedical sector and mainly focused on tools of diagnosis, prognosis, patient management and telemedicine. The major problem of this developments the emerging of system for real time response.

❖ Biomedical Applications withUsing Embedded Systems by GulcicekDere( 2021) ( WoS)
In this paper, disclose the design and development tools of embedded system in biomedical sector and it deals with tools development of safety healthcare of people in the event of epidemic infectious diseases in process.

❖ An eye-tracking based robotic scrub nurse: proof of concept by Ahmed Ezzat· Alexandros Kogkas and teams. (2021) (Springer)
This paper discusses the surgery assistive robotic devices and its acted as a third hand to achieve the various tasks and it's to be control the wearable eye tracker glass which is integrated with the motion sensor and the motion of eye ball movement it to activate or pass the command to the ASD, according to that command it will perform.

❖ Robot, Pass Me the Scissors! How Robots Can Assist Us in the Operating Room by Juan P. Wachs, Purdue University, West Lafayette, IN 47906, USA (2012) (Springer).
This paper describes the lack of nurse availability in hospitals especially they need well experience nurse at the time of surgery. So resolve the problem they are focused to design the robotic assistance in OR and it includes an assistive robot for object picking, haptic feedback controlled robots which are controlled by the gesture controlled mechanism.

❖ Surgical Instrument Handling and Retrieval in the Operating Room with a Multimodal Robotic Assistant By Mithun G. Jacob, Yu-Ting Li, Juan P. Wachs( 2013) (IEEE) .

This paper considers the design and development of robotic scrub nurse (RSN) designed for human safety -robot collaboration in the operating room (OR) and it assist the surgical staff in the OR by Delivering instruments to the surgeon and operates through a multimodal interface allowing instruments to be requested Through verbal commands or touch less gestures control method.

❖ A Novel Gesture Recognition System for Intelligent Interaction with a Nursing-Care Assistant Robot by Geng Yang and team (2018) ( MDPI – Scientific Journal)

This paper discussed a nursing-care assistant robot prototype which is to be developed based on the (HMT) gesture recognition system. The HMT consist of data acquisition, data processing, and a natural a human robot interface for the smart infrastructure.

**PROBLEM IDENTIFICATION**

Now days a different number of robotic systems are available in hospitals for different kind of application, but no one can assist a surgeon during at the time of surgery for delivering the needful tools according to the surgeons needs. For delivering the tools at the time of surgery the surgeons need a personal assistant nursing system. In this case, the surgeons need in well experience nurse with the knowledge of surgical tools and its handling methods and also need to focus in multiple tasks related to surgery.

In these cases, the surgeons need to faces the three major problems.

1. Availability of experience nurse.
2. Communication problem (Language, pronunciation, command delay)
3. Manmade error.

**OBJECTIVE:**

This research is to develop a robotic system, which can assist surgeon during surgery time for delivering the tools and monitoring the health parameters to avoid the mentioned problems. Today, a lot of medical robot system is available but none can assist a surgeon during a complex surgery. So surgical assistance robot system has been designed for this purpose. It can understand the surgeon's needs by them inputs and bring the appropriate surgical tool during surgery and its continuously monitoring the health parameters of patient. Also getting the details from the environmental data during the surgery. The robot can understand and fetches the necessary tools and respective necessary things like cotton pads and clamps to stop bleeding, fetches scissors, catheter, and needle etc. These all the functions will be achieved by the MSN network and AI technologies.

**EXISTING SYSTEM**

The existing systems of Assistant Nurse Systems are developed in different methods for different kind of applications (Refer Figure 1).

The lists of bio medical robotic systems are,

**Electronic Medical record robotic system:** In healthcare sectors this types of robotic systems are used to maintain and monitoring the patient health conditions and it maintain these recordsand medical history about the patient for further treatment.

**Medication Management robotic system:** This robotics system specifically designed for patient and patient care person to manage maintain the exact medicine list for a treatment.

**Patient Monitoring robotic system:** This type of robotic systems which is used to maintain the monitor the patient health condition in hospital or remotely. Based on this monitoring system the doctors can easily monitor the patient health condition and easily can take prevention action to improve the patient health condition.

**Scrub Assistance Robotic system:**

- Used in surgery as an assistant.
- It assists the surgical staff in operation room by delivering the tools to surgeon. The surgical assistant robots are mainly followed the below mentioned method for its development.

The surgical assistant robotic (SAR) system mainly classified into two types based on the input accessing methods: (Refer Figure 1)

1. Contact Method
2. Touch Less Method

**Contact Methods**

The control functions of the system to be defined according to the contact of event or movement. Based on the event, the function to be implemented by assigned program (Refer Figure 2)**.**

Ex: **Eye ball/ Eye Track Robotic system:**

This robotic system mainly consists of camera with sensors, robotic arm system, and which is controlled and monitor by the software program. The camera systems continuously monitor the human eye ball contact movement. According to the eye ball movement the robotic arm to be controlled. This method of technology involved in various applications like assistive device for disability people, etc.

Here the figure 2&3 discuss about the function flow of eye ball tracking systems.

In this system the eye ball tracker mounted with wheelchair and it to be controlled by the controller by defined software program [3]. The robotic arm also controls by the eye ball movement for the user defined functions.

**Touch less Method**

This method of robotic system has a capability to interact with objects, surfaces, or their environment without physically making contact (Refer Figure 3).

Ex: Gesture control Robotic system

A gesture control robotic systems allows robots to interpret and reply to human gestures as instructions or commands.

The figure 3 discuss about the gesture control system based on the hand movement for the robotic system to move Up, Down, Right and Left, Hand Grip Close and Hand Grip Close.

This technology allows the users to interconnect with the robots instinctively using hand gesture movement, body language or some special gestures to communicate the actions or directives to the robot.

**IMPLEMENTATION METHOD:**

The Surgical Assistant Robot System is mainly developed in to assist the surgeon in Operation room and it also monitor the patient health condition and environmental situation by the using the MSN (Multisensor Network).

This robotic system can develop in 2 main methods.

1. Verbal Communication
2. Non Verbal Communication

Verbal and non-verbal method of communication plays a vital role in the robotic functions.

**Verbal Methods**

This method of robots involves the use of spoken language or synthesized speech to convey the information by the given instructions, responding to queries, or expressing status updates audibly by voice command.

**Speech Recognition:** Robots can understand and respond to spoken commands with natural language, enabling more intuitive interaction.

**Text-to-Speech (TTS):** Robots equipped with TTS technology can convert written text into spoken words, allowing them to communicate information or instructions verbally.

**Voice Feedback:** Robots can provide feedback or updates on their status, actions, or any issues using verbal communication. Throughout this method the user can understand the robotic functions.

**Non-Verbal Methods**

In this method the information and command to be transfer through the various non-verbal cues, like body language, face expressions, gesture control, postures control functions without the use of voice and sound commands.

**Gesture Recognition:** Robots can interpret human gestures as commands. This involves using cameras or other sensors to recognize and understand hand movements, body language, or facial expressions.

**Facial Expression Recognition**: By analyzing facial expressions, robots can gauge human emotions and respond accordingly. This is particularly useful in social or caregiving robots.

**Body Language Interpretation:** Understanding and responding to human body language allows robots to interact more naturally with users, providing a more intuitive and user-friendly experience.

**Visual Feedback:** Robots can use lights, displays, or other visual cues to convey information or responses to users. This could include indicating their status, acknowledging commands, or signaling errors.

**Haptic Feedback:** While not strictly non-verbal, haptic feedback involves using touch or force feedback to communicate with users. For example, a robot might provide a gentle vibration to confirm a successful action.

**Combining Verbal and Non-Verbal Methods**

**Multimodal Interaction:** Many advanced robotic systems integrate multiple communication modalities simultaneously. For instance, a robot might combine speech recognition with gesture recognition to enhance user interaction and understanding.

In this research work the Non Verbal Communication method to be used for implementation because can easily avoid the communication error and issues.

In this assistant surgical robotic systems to be implement by the **touchable keypad of Non-verbal communication** for the robotic functions.

There are different types of method to be used for robotic function as discussed in earlier part. But all these method of communication system produces the errors based on Communication problems like language, pronunciation, command delay and manmade error.

In this touchable keypad based system of Non-verbal communication method system produces high accuracy in data transmission of information of command for the robotic functions.

**Functional details of block diagram:**

In this surgical nurse assistant robotic system mainly consist of the following blocks (Refer Figure 4).

1. MSN MODULE (Patient Health Monitoring and environmental monitoring.)
2. Touchable Keypad (For I/P control signal for robotic control system).
3. AI based controller system for Robotics.
4. Robotic Arm System.
5. Monitoring and Alerting system.

**MSN Module**

The multi-sensor network (MSN) module consists of multiple sensors connected in single controller. Here this sensor is used to sense the major parameter (SPo2, Blood Pressure and sugar, EGC) of patient health condition at the time of surgery to avoid the critical situations and it also sense the external environmental parameters ( temperature, air quality of $O_2$, and humidity level) of surgery room (Operating Room) .

**Touchable Keypad**

This touchable keypad system to be functioned based on the non verbal communication method. Based on this method can avoid the communication and man made errors. Based on the key selection the user can control the robotic arm function for the tools delivery according to the type of surgery and it also used to check the patient health conditions. The entire system function will be selected by the key selection from keypad. According to the key pressing the control system to be activate the respective function.

**AI based controller system for Robotics**

The system controller used to controller the user defined function according to the patient health and environmental monitoring system. For robotic arm control function to be implemented though the controller for the user function. The AI technology is also included for the decision making for the surgical tools arrangement in the tray according the respective surgery. Here the AI technology is to be implementing for the tools rearrangement in the surgical tools box tray [4]. Initially this system selects the type of surgery through keypad. After selection of surgery type the system needs to check the tools box tray whether the needed tools to be placed in the respective slot otherwise it to be collect the needful tools and arrange it in respective slots.

**Robotic Arm System**

A robotic arm system designed as a surgical assistant nurse to deliver surgical tools is an innovative application of robotics in the medical field. This kind of technology aims to enhance surgical procedures by providing precision, efficiency, and controlled assistance. Here are some key features and benefits of such a system:

**Precision and Accuracy:**

The robotic arm system can offer high levels of precision in tool delivery, minimizing the risk of human error during surgery. It can be programmed to follow specific way, to ensuring accurate placement of surgical instruments.

**Efficiency and Speed:**

The robotic assistant nurse can work seamlessly with human surgical teams, serving to streamline the surgical process. Automated movements and fast response times contribute to the efficiency of the surgical function.

**Minimally Invasive Surgery:**

Robotic systems are often designed to facilitate simply invasive measures, reducing the size of incisions and promoting faster recovery for patients. Smaller incisions can result in less pain, decreased risk of infection, and shorter hospital stays.

**Enhanced Visualization:**

Integration of robotic systems may include advanced imaging technologies, providing surgeons with enhanced visualization of the surgical site.

**Reduced Fatigue:**

Robotic assistants do not experience physical fatigue, ensuring consistent performance throughout lengthy surgical procedures.

This can contribute to maintaining a high standard of care and focus during the entire surgery.

**Adaptability:**

The system can be adaptable to various surgical specialties allowing its use in a wide range of procedures.

Modular designs may allow for upgrades and customization based on evolving surgical needs.

Safety Features:

Incorporation of safety mechanisms, such as collision detection and avoidance, ensures that the robotic arm operates without causing harm to patients or surgical teams. While the development and use of robotic surgical assistants continue to evolve, the goal is to improve patient outcomes, reduce recovery times, and enhance the overall quality of healthcare delivery in surgical settings.

**Monitoring and Alerting system**

The monitoring and alert system is used to monitor the patient health condition and environmental conditions at the time of surgery.

The monitoring system will monitor the patient health parameter and environmental condition data through the different sensor and this parameter range to be calibrated in controller. If the received parameter is beyond the set value the alert system to be alerts the surgeon. Based on the alert surgeon can avoid the errors and save the patient health conditions.

**Functional Flow Diagram**

The system function diagram explains the functional flow details of the surgical assistant nursing system (Refer Figure 5).Initially the system initializes the robotic control and monitoring system function. These functions are split into two sections.

i) Robotic Arm control system
ii) Monitoring system

**Robotic Arm control system**

The Arm control system initially activating the self-testing condition of hand movements for surgical tools delivering and pick up. After the self-testing the surgeon selects the type of surgery by selection the key from the keypad. Initially some pre-set function to be implemented for surgery type and its needful tools then its position in tools box. According to the selection of surgery type the robotic system check the tools box for availability of tools and position [5]. The tools availability of tools and its positions as in pre-set function the arm system to be ready for surgery for delivering and receiving the tools according to the surgeon need. (I/P instruction given through keypad). If the tools availability and its positions are not in the pre-set function, immediately the arm system which will collect the needful tools in the main tools box and it rearrange the tools according to the pre-set function [6].

The AI system function to be implemented for the functions of tools identifications and its rearrangements according to the type of surgery.

**Monitoring and Alerting system**

This system to be implemented for monitoring the operating room environmental condition and patient health condition based on Multisensor network module. The MSN module parameter to be monitoring and it compare with the pre-set parameter. If the received parameter is beyond the pre-set parameter, it will immediately activate the alert system [8].

## RESULT AND CONCLUSION

In the surgical nursing assistant robotic system mainly implemented by the combination of MSN module, Robotic Arm system with AI. These technology is used to avoid the manmade error and time delay of tools delivery at the time of surgery as well as this system also reduce the man power (experience nurse). This surgical assist robotic is a suitable replacement for the surgical assistant nurse. The AI technology integrated into the robotic system allows for adaptive learning, where the robot can improve its tools arrangements and delivery processes over time by analyzing past surgeries and outcomes and also the AI – driven system provides high precision in delivering the correct tools to the surgeon, minimizing delays and optimizing the flow of surgical procedures. The potential for future enhancements is vast, including the use of machine learning to facilitate more complex tasks and the integration with other robotic systems for a fully automated surgical environment.

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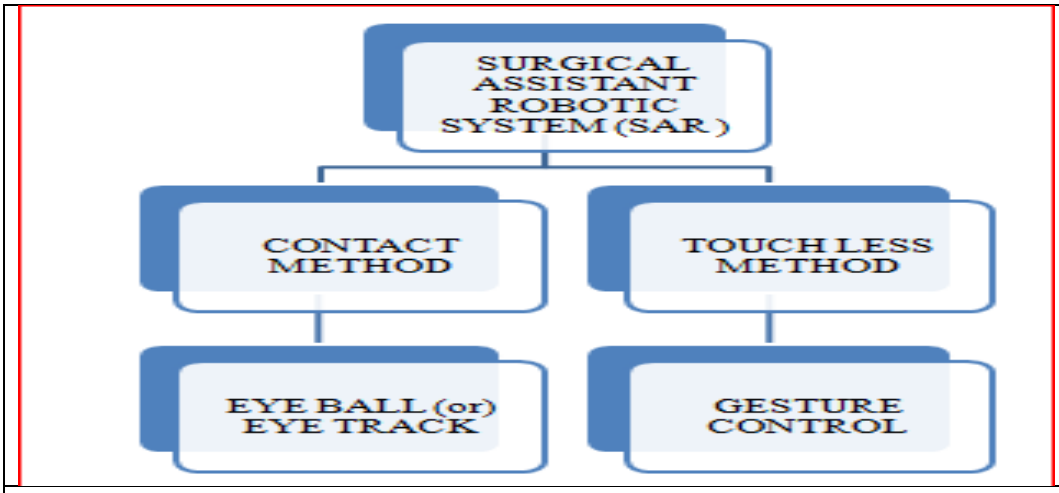


**Figure 1: Different types of SAR system with its methodology.**

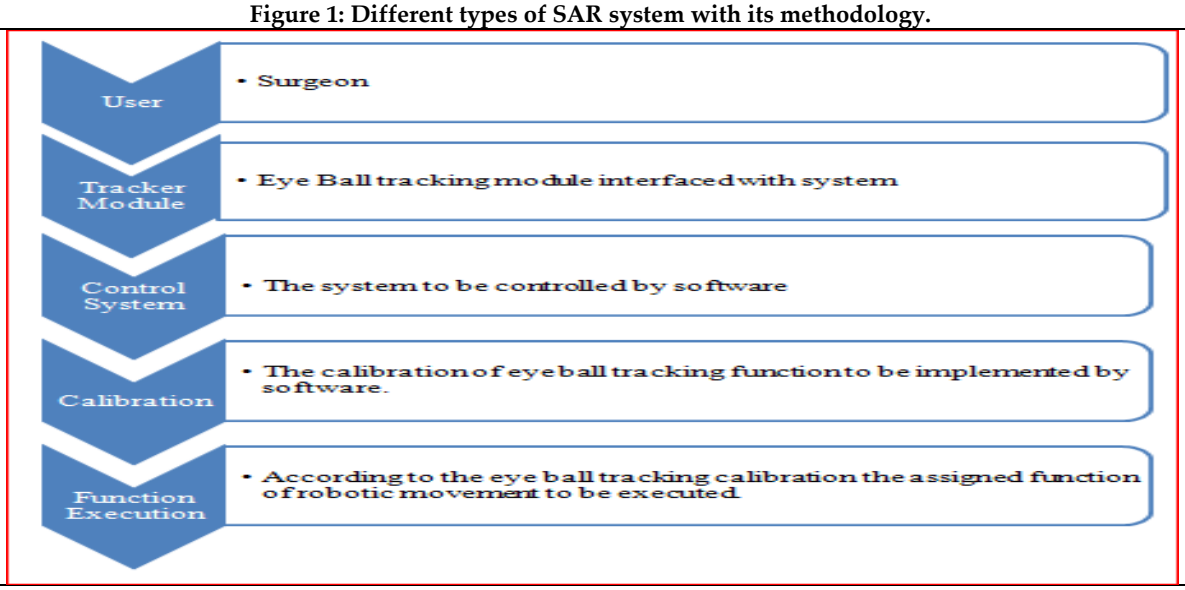


**Figure 2: Flow chart for function execution.**

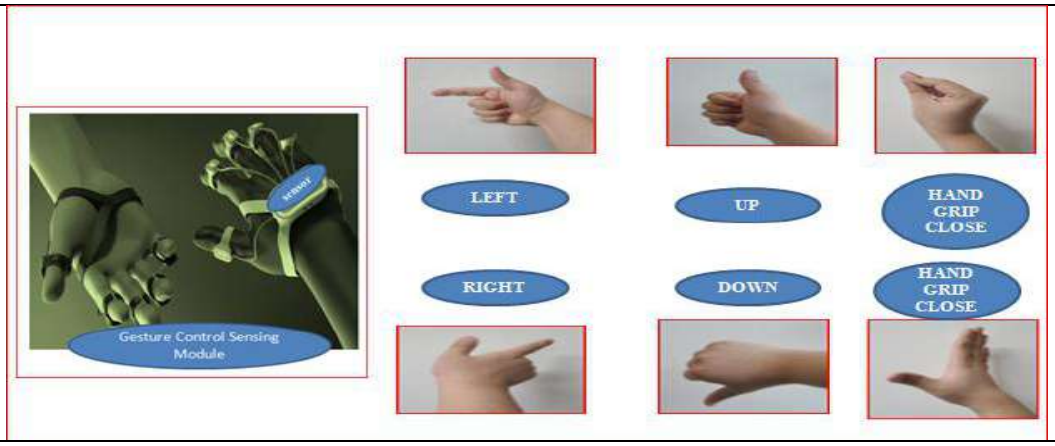


**Figure 3: Gesture Control Robotic System based on hand movement.**

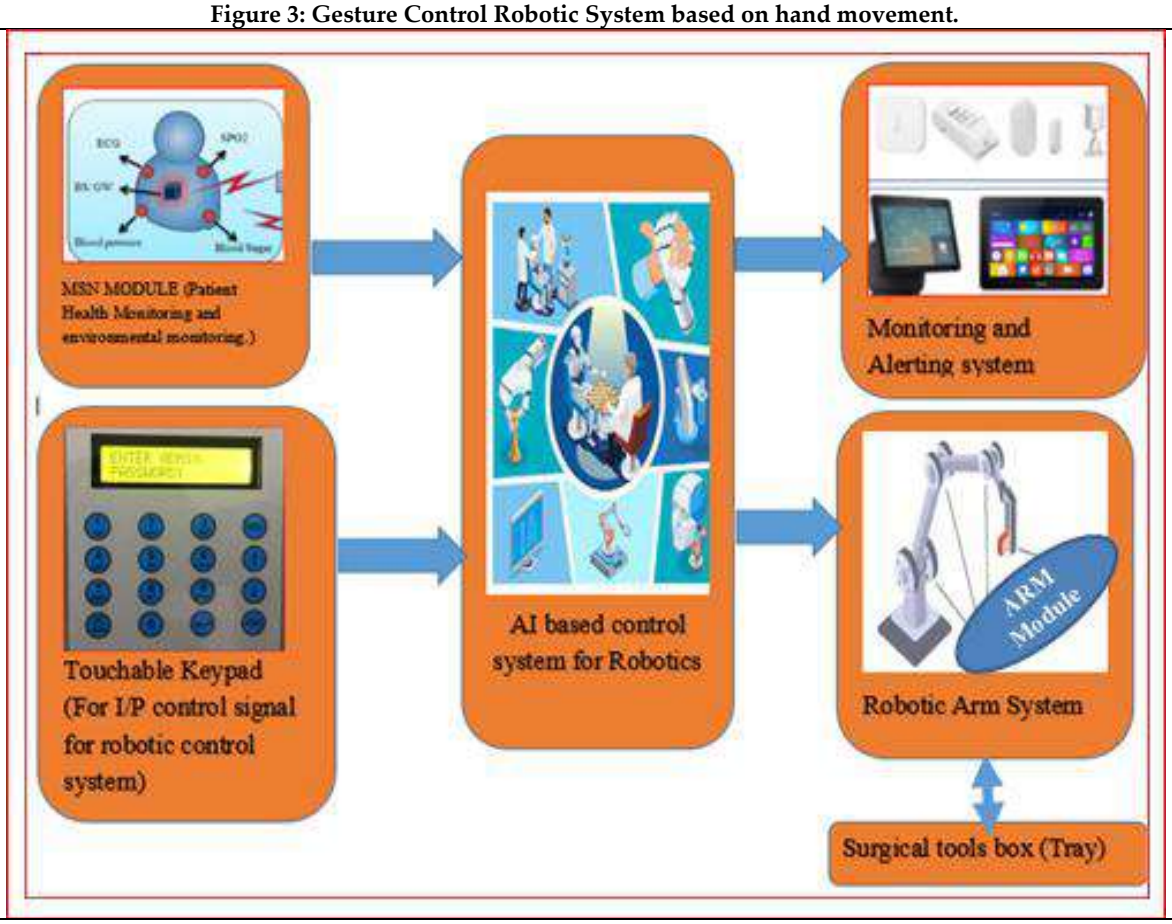


**Figure 4: System Block diagram**

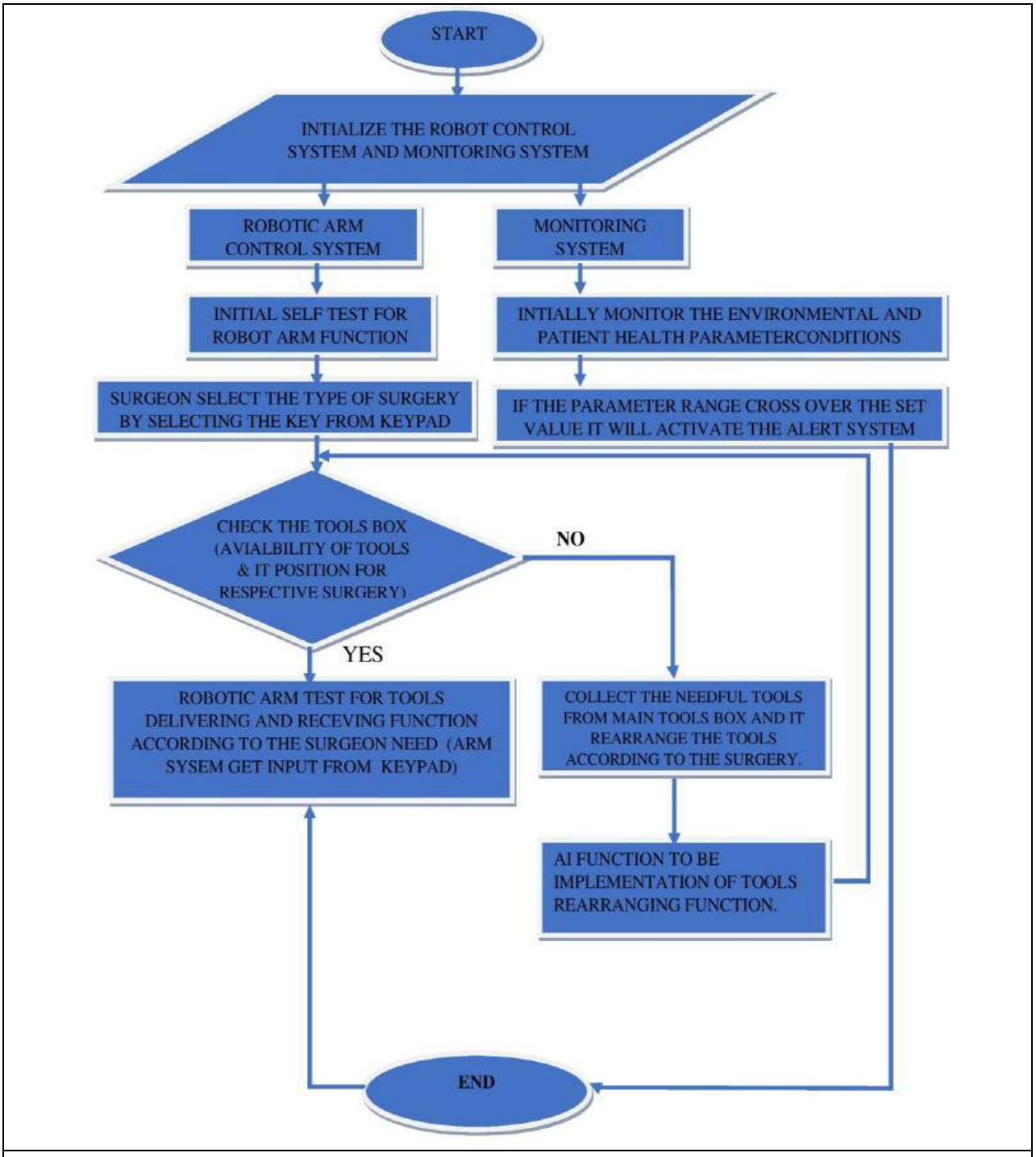


**Figure 5: System functional flow diagram**

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**RESEARCH ARTICLE**

# Heavy Metal Analysis of Siddha Poly Herbal Formulation Karanthai Chooranam

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## ABSTRACT

Heavy metal analysis, phytochemical screening, and aflatoxin estimation were carried out in Karanthai Chooranam, a Siddha polyherbal formulation. This study examines the nature of raw ingredients, which were properly sourced, purified, and processed through traditional Siddha methods. Atomic Absorption Spectrometry (AAS) used for the analysis of heavy metals showed that mercury, lead, and cadmium levels were all below the detectable l imit. The arsenic content, on the other hand, was found to be at 0.70 ppm, thus far from the permissible limit of 3 ppm. Phytochemical evaluation by TLC and HPTLC revealed six major phytochemical peaks. Among these, peaks 5 and 6 represented the most significant bioactive compounds. Results of aflatoxin analysis indicated minimal presence of Aflatoxin B2 0.11 ppm, within the permissible limit of 0.1 ppm, while Aflatoxins B1, G1, and G2 were not detected. From these results, Karanthai Chooranam can be characterized as rich in a complex phytochemical profile that suggests therapeutic potential, while being safe regarding heavy metals and aflatoxins. Thus, the study suggests that this

formulation can be safely used in traditional Siddha medicine and consumed for its medicinal properties. Further research is recommended to correlate the phytochemical profile with therapeutic efficacy.

**Keywords:** *Karanthai Chooranam*, Standardization, Herbal Drug, Physicochemical, Phytochemical

## INTRODUCTION

India is a mother hub for the development of Ayurveda, Unani, Siddha, Homoeopathy and other natural herbs-based health science (Ayush). Ayush Pharmaceutical industry is having great potential and opportunities for development in future. Mainly in following herbal medicinal plants and their value-added products well accepted in domestic and international market [1]. Siddha system of medicine, which is the traditional heritage science of Tamil origin, renders the application of numerous medicines based on plants, minerals-metals and animal origin [2]. World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles6. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose [3]. Herbal medication as the major remedy in the traditional system of medicine has been used in medical practices since antiquity. The Siddha system is one of the traditional systems of medicine which is flourished in southern India, especially Tamil Nadu. In the Siddha system besides herbs, metals and mineral drugs were used as medicine in 32 subdivisions [4]. The mode of preparation and plant used in traditional medicine varies from place to place. The traditional system of medicine became significantly more popular all over the globe because of its curative property, less toxicity and minimal side effects. Hence most of the Siddha formulations are herbal and polyherbal formulations. Central Council for Research in Ayurveda and Siddha has provided proforma for each category of medicine [5]. Standardization of formulation was done based on its macroscopic and microscopic characters, Physico-Chemical Parameters, Phyto Chemical Screening, Fluorescence studies and Thin Layer Chromatography Analysis. Phytochemical screening has shown the presence of Alkaloids, Glycosides, Saponins, Phytosterols, Fixed Oil, Resins, Phenols, Flavonoids and Tannins. ochemical, Phytochemical

## MATERIALS AND METHODS

**Selection of Source of Drugs**

The raw drugs were procured from the raw drug shop R.N. Rajan and Co, Chennai. After proper authentication by the Medicinal Botanist, Govt. Siddha Medical College, Arumbakkam, Chennai, the preparation was made.

**Purification of Raw Drugs**

All herbal drugs are purified as mentioned in the text Gunapaadam Mooligai Vaguppu[7].

**Ingredients [8] Table 1.**

**SOP for *Karanthai Chooranam* Procedure**

All the raw drugs were purified as per traditional text and powdered followed by the procedure*Vasthrakayam*to get a fine powder. The final product was stored in an air tight container.

**Heavy Metal Analysis [16 – 17]**

Methodology Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item. Sample Digestion Test sample was digested with 1mol/L

HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3. Standard reparation As& Hg- 100 ppm sample in 1mol/L HCl Cd &Pb- 100 ppm sample in 1mol/L $HNO_3$

**TLC AND HPTLC ANALYSIS [18].**

**TLC Analysis**

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Toulene: Ethyl Acetate: Acetic Acid (1.5:1:0.5) After the run plates are dried and was observed using visible light Shortwave UV light 254nm and light long-wave UV light 365 nm

**High Performance Thin Layer Chromatography Analysis**

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantitation of Nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials. Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of Phyto constituents present in each extract and Rf values were tabulated.

**AFLO Toxin Assay**

Standard Aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

## RESULTS

Determination of heavy metals in polyherbal formulation is one of the major concern for consumption which are prone to adulteration and contamination. The heavy metal analysis of *Karanthaichooranam* reveals that the presence of heavy metals like Mercury, Lead, Arsenic and Cadmium are below detected limit as per *AYUSH* (Table 2).

**TLC and HPTLC Analysis**

The Peak Table of HPTLC finger printing analysis (picture 4) of the sample KC reveals the presence of six prominent peaks corresponds to presence of six versatile Phyto components present within it. Rf value of the peaks ranges from 0.12 to 0.74. Further the peak 5 and 6 occupies the major percentage of area of 44.39 and 26.95 % which denotes the abundant existence of such compound. Followed by this peak 2 and 3 occupies the percentage area of 10.03 and 8.87%. Aflatoxins are poisonous secondary metabolites found in contaminated herbal drugs. The result reveals that there was no spots were identified in the test sample loaded on TLC plates with respect to Aflatoxin B1, G1 and G2 when compare to standard. Whereas presence of Aflatoxin B2 were identified. This level of B2 seems to be within the limit prescribed by the AYUSH (Table 3).

***DISCUSSION***

The heavy metal analysis and phytochemical evaluation of Karanthai Chooranam, a Siddha polyherbal formulation, provide critical insights into its safety, efficacy, and quality. The study aimed to assess the levels of toxic heavy metals, identify phytochemicals, and detect potential aflatoxin contamination to ensure the formulation meets safety and quality standards.

**Heavy Metal Analysis**

The analysis of heavy metals in Karanthai Chooranam revealed that the concentrations of mercury, lead, arsenic, and cadmium were either below the detection limit or within the permissible limits established by AYUSH standards. Specifically, mercury and lead were below detection limits, which indicates that the formulation is free from these toxic elements. Arsenic, though detected, was found at a concentration of 0.70 ppm, well below the maximum limit of 3 ppm. Cadmium was present at 0.005 ppm, a value that is significantly lower than the detection limits for this metal.

These results are particularly reassuring because heavy metal contamination is a common concern in herbal medicines, which can have serious health implications if not controlled. The low levels or absence of these metals in Karanthai Chooranam suggest that the formulation is safe for use from a heavy metal toxicity perspective. This is consistent with traditional Siddha practices that emphasize careful preparation and purification of raw materials to avoid contamination.

**Phytochemical Analysis:** The Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) analyses were instrumental in profiling the phytochemicals present in Karanthai Chooranam. TLC provided an initial qualitative assessment of the phytochemical profile, revealing distinct spots indicative of the presence of various compounds. The HPTLC analysis, on the other hand, offered a more detailed and quantitative view of the phytochemical constituents. The presence of six prominent peaks, with Rf values ranging from 0.12 to 0.74, suggests a complex mixture of bioactive compounds. Peaks 5 and 6, which occupy significant percentages of the chromatographic area (44.39% and 26.95%, respectively), likely represent the major active constituents in the formulation. The identification and quantification of these peaks can help in understanding the formulation's therapeutic efficacy and ensure consistency in quality.

**Aflatoxin Analysis**

Aflatoxins are carcinogenic compounds that can contaminate herbal medicines, posing a significant health risk. In this study, the absence of aflatoxins B1, G1, and G2, with only a minimal presence of aflatoxin B2, is noteworthy. Aflatoxin B2 was detected at 0.11 ppm, which is just above the permissible limit of 0.1 ppm. However, this level is still relatively low and does not pose a significant health risk, especially when compared to higher permissible limits in other pharmacopoeias.

## CONCLUSION

The comprehensive analysis of Karanthai Chooranam confirms that the formulation is within safe limits for heavy metals and aflatoxins, supporting its safety for consumption. The detailed phytochemical profile obtained through TLC and HPTLC underscores the formulation's complexity and the presence of potentially therapeutic compounds.

These findings not only validate the traditional preparation methods and safety standards but also provide a foundation for further research into the efficacy and quality control of Siddha formulations. Future studies could focus on correlating the phytochemical profile with specific therapeutic outcomes and exploring the mechanisms of action of the major constituents identified in this study. Overall, Karanthai Chooranam demonstrates a promising profile, supporting its continued use in traditional Siddha medicine with assured safety and efficacy.

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**Table 1. Ingredients**

| S.No | Ingredients | Quantity (grams) |
|---|---|---|
| **1** | *Spheranthus indicus* | 1 *Balam(35 grams)* |
| **2** | *Nigella Sativa* | 1 *Balam(35 grams)* |
| **3** | *Terminalia bellirica* | 1 *Balam(35 grams)* |
| **4** | *Acorus calamus* | 1 *Balam(35 grams)* |
| **5** | *Piper longum* | 1 *Balam(35 grams)* |
| **6** | *Piper nigrum* | 1 *Balam(35 grams)* |

| | | |
|---|---|---|
| **7** | *Celastrus paniculatus* | 1 *Balam(35 grams)* |
| **8** | *Rock Salt* | 1 *Balam(35 grams)* |
| **9** | *Costus speices* | 1 *Balam(35 grams)* |
| **10** | *Zingiber offoicinale* | 1 *Balam(35 grams)* |
| **11** | *Celrotendrum serratum* | 1 *Balam(35 grams)* |
| **12** | *Psoralea corylifolia* | 1 *Balam(35 grams)* |
| **13** | *Plumbago zeylanica* | 1 *Balam(35 grams)* |
| **14** | *Terminalia chebula* | 1 *Balam(35 grams)* |

**Table 2: Shows the heavy metal analysis of *Karanthai Chooranam***

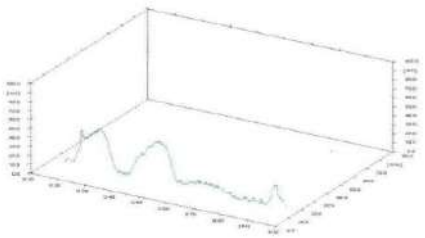
| **Name of The Heavy Metals** | **Absorption Max Λ Max** | **Result Analysis** | **Maximum Limit** |
|---|---|---|---|
| **Mercury** | 253.7 nm | BDL | 1 ppm |
| **Lead** | 217.0 nm | BDL | 10 ppm |
| **Arsenic** | 193.7 nm | 0.70 | 3 ppm |
| **Cadmium** | 228.8 nm | 0.005 | - |

**Table 3 shows the Aflatoxin level in *KaranthaiChooranam***

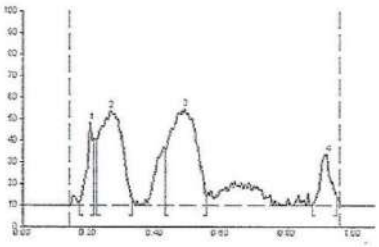
| Aflatoxins | Presence | Limits |
|---|---|---|
| **Aflatoxin B1** | Not Detected | 0.5 ppm |
| **Aflatoxin B2** | 0.11 ppm | 0.1 ppm |
| **Aflatoxin G1** | Not Detected | 0.5 ppm |
| **Aflatoxin G2** | Not Detected | 0.1 ppm |

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.17 | 2.2 | 0.21 | 38.3 | 25.39 | 0.22 | 31.1 | 632.5 | 9.16 |
| 2 | 0.23 | 31.0 | 0.27 | 43.9 | 29.06 | 0.33 | 2.7 | 2503.0 | 36.26 |
| 3 | 0.43 | 25.8 | 0.49 | 45.0 | 29.80 | 0.56 | 3.8 | 3114.2 | 45.11 |
| 4 | 0.88 | 0.1 | 0.92 | 23.8 | 15.75 | 0.95 | 5.0 | 654.0 | 9.47 |

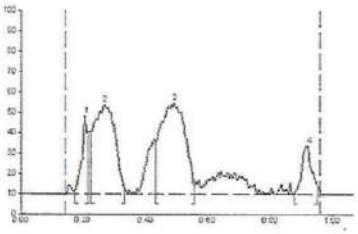
**Picture 1 Shows TLC Analysis at 366 nm**



**Picture 2 Shows Track at All Wave length**



**Picture 3 Shows HPTLC finger printing of Sample *Karanthai chooranam*(KC)**



**Picture 4 Shows Peak Table**

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**REVIEW ARTICLE**

# A Review on the Effect of Advanced Glycation End Products on Adolescents

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## ABSTRACT

Purpose: Advanced glycation end products (AGE) are a group of amalgamated compounds of protein and fat possessing various deleterious effects including non-communicable diseases. Adolescents are exposed to high serum AGE levels due to the rapid consumption of junk foods. Hence, this category of the population is at an increased risk of developing metabolic illnesses in the future. Methods Literature search was done on electronic databases like PubMed and Scopus employing keywords such as AGE and Adolescents, AGE and Obesity, AGE and Diabetes mellitus etc. A total of 117 articles were looked upon and after assessing for eligibility 14 articles were included as part of the review. Results AGE had shown to possess harmful effects on the adolescent population by the development of chronic metabolic diseases with the focus on diabetes mellitus, Obesity, and abnormal thyroid functioning. It was also observed that incidences of obesity reported among adolescents showed elevated serum AGE and Serum CML levels predisposing to the fact that AGE can be trapped in the fat stored cells or tissues. Exogenous or dietary AGE had an additional load on the AGE pool in vivo leading to the occurrence of various metabolic diseases. Few studies have shown the dependency of age and presence of obesity as risk factors for the development of AGE among adolescents. Conclusion Limited number of studies have focused on the impact of AGE among adolescents and hence, this review throws light upon the harmful effects of AGE in the context of adolescent health.

**Keywords:** Advanced glycation end products, Obesity, Diabetes mellitus, Adolescents, Carboxymethylysine, Exogenous.

# INTRODUCTION

Advanced glycation end products (AGEs) are a diverse group of compounds produced when reducing sugars react with amine groups linked to proteins, nucleotide bases or fatty acids, forming glycosylated products.[1] AGEs are produced by Maillard reaction were the mixtures of amino acid and sugar become brown upon heating.[2]. Maillard reaction (non-enzymatic glycation or browning) in foods has been well studied by the food industry to control food quality. However, it is only 40 years ago that a similar glycation process was recognized in human body by the observation of increased formation of glycosylated haemoglobins in diabetic patients [3] which leads to the formation of detrimental advanced glycation end products (AGEs) in humans.[4] In addition to AGE's being derived from within the body, AGEs also exist in foods. Uncooked animal derived foods and foods cooked at higher temperature have naturally present AGEs [5]. Exogenous dietary AGEs have been suggested to increase the in vivo pool of AGEs after intestinal absorption, and contribute to the development of diabetes and related complications. [6] The adolescence period witnesses multiple changes ranging from physiological changes- Rapid growth spurt, Puberty, Psychological changes- Thought process and Psychosocial changes- Personality and attitudes. Behavioural establishments also occur during this transitioning age, where eating choices are also established. [7]

Adolescents consume approximately 20% of their daily energy intake as snacks, with salty and sweet foods contributing a majority of the snacking calories directly proportional to the development of overweight and obesity status among them. Alarming increases are observed in the prevalence of obesity in children, with 32% of children and adolescents in the United States at or above the eighty-fifth percentile. [8]. In a study conducted in India to determine the prevalence of obesity among children and adolescents, it was noted that higher rates of obesity were prevalent among the adolescents than children. [9]. The consumption of processed foods is increasing among adolescents which is associated with increased exposure to dietary advanced glycation end products hence putting them at an increased risk of metabolic and cardiovascular events in the future. [10]. Thus, an interest in adolescent nutrition and health has expanded rapidly in recent years, in part due to the realization that investing in adolescent health, nutrition, and well-being may accelerate progress toward the Sustainable Development Goals.[11] Limited studies have focussed on the impact of AGE on the adolescent population, providing an area of interest yet to be established. Hence, the objectives of the current review is to explore the effect of advanced glycation end products on adolescent health and also highlight upon the metabolic complications which occur as a cascading effect of AGE on the future generation of the country.

**Methods**

**Search Strategy**

A thorough search on electronic databases like PubMed and Scopus were conducted. The time period of the articles was restricted to 10 years. The keywords used for the article search were as follows:- Advanced glycation end products and obesity, Advanced glycation end products and Type I Diabetes mellitus, Advanced glycation end products and Adolescents, Advanced glycation end products and insulin resistance, AGE and Adolescents, AGE and Obesity, AGE and Type-I DM, AGE and Insulin resistance, Dietary AGE and adolescents, CML and Adolescents, Fluorescent AGE and adolescents, Carboxymethylysine and Obesity, CML and obesity, Carboxymethylysine and Insulin resistance, AGE and metabolic syndrome. All English language articles were included.

**Ethical Issues**

Not applicable

**Selection Criteria**

Studies involving the harmful effects of AGE on adolescent health and metabolic diseases per se were included. The type of articles was restricted to invitro studies, original articles, in vivo and human studies. Research Note and commentaries were excluded from the search. Few articles were excluded as they did not meet the eligibility criteria. A schematic representation of the articles selected had been mentioned in *figure I*.

**Impact of Advanced Glycation End Product on Adolescents**

Adolescence is a period of rapid growth and psychological changes. During this age, independent decisions regarding social groups, behavioural establishments and food choices occur.[12] The environment of the adolescents plays a major role for choosing the right foods for consumption. In the recent years, various Food Swamps are located at proximity in campuses of schools and other educational institutions, which provide means of consuming these unhealthy processed foods. [13] Dietary Advanced glycation end products primarily found in foods subjected to high temperature cooking techniques i.e., processed and junk foods have deleterious effects on the metabolic parameters among individuals. The consumption of highly processed foods are critical among the adolescents making them exposed to a series of lifestyle diseases. Table I illustrates the studies in relation to the presence of AGE and its effect on the adolescent health. It was observed, that increased levels of fasting glucose, Triglycerides, LDL and Blood pressure were present among adolescents with an elevated dAGE consumption indicating a pathophysiological relationship between dAGE and metabolic parameters. (Fig II)

A study done on seventy-four overweight adults who were administered to consume a low AGE diet for a duration of 4 weeks had decreased levels of fasting glucose and insulin levels compared to the high AGE diet group, proving a direct relationship between exogenous AGE consumption and insulin resistance.[14] A similar study found an interesting theory correlating the level of FT4 and Serum AGE in adolescents showing a positive significance between these components. Hence, this acts as evidence to prove the improper functioning of the thyroid gland can be attributed to elevated glycated end products in the blood.[15] Due to the AGE-RAGE interaction, various compounds such as Interleukin-6, Tumour necrosis factor alpha (TNF) are expressed leading to proliferation, cell differentiation and cell mitosis and hence causing tremendous changes in the cell membrane which can directly have an impact on the thyroid gland. [16] Adolescents are at higher risk of AGE compared to adults as reported by increased serum CML levels. Obesity was shown to have a larger contribution towards a steady increase in AGE due to the presence of inflammation and trapping of CML in the adipose tissues. [17]. Very limited studies have highlighted the effect of AGE among adolescents, predisposing them to develop metabolic complications in the future.

**Effect Of Diabetes Mellitus on Advanced Glycation End Products**

Table II represents the articles on advanced glycation end products and metabolic complications. Adolescents diagnosed with Type-I DM had increased odds of dramatically high serum AGE levels as compared to healthy individuals. This is in accordance to a mechanism which directly affects the insulin hormone causing an upregulation in the glucose uptake thereby leading to hyperglycaemia. Another theory postulates, the overexpression of RAGE and SIRT1 in the presence of AGE which causes changes in the insulin leading to diabetes mellitus.[19] An interesting observation was noted in adolescents where, an increase in age had a direct effect on the AGE Levels. *Galler et al, 2003* studied that adolescents in the age group of 16 years had elevated AGE levels than adolescents in the age group of 13 years and below. In addition to Age, serum Lipid, BMI and Weight also had a directly proportional relationship with AGE among the adolescents. [20] Previous literature has highlighted the nature of hba1c which serves as an indicator for glycaemic monitoring and is also regarded as a glycated compound thus possess a history with AGE.[21] *Kostolanská et al, 2009* determined significant correlations between hba1c and serum AGE in a group of poorly controlled diabetic adolescents indicating oxidative stress and glycation end products as contributors towards diabetic complications in the future. [22]

A study implicated the various risks involved around high AGE levels which directly cause microvascular complications such as retinopathy, nephropathy, and neuropathy among adolescents. [23] Studies have also observed differences between AGE levels in obesity and type-1 DM individuals where a marked degree of

comparison was observed between both the groups. The Type-1 DM adolescents had elevated AGE levels than the obese adolescents, which showcased high cardiac parameters in the latter. Gender also played a pivotal role in AGE measures. The Female population was noted with elevated AGE levels than the males. This can be attributed to the fat percentage usually higher among the female population. [24]

**Effect Of Obesity on Advanced Glycation End Products**

The formation of AGE is accelerated in various inflammatory conditions, the primary example including obesity. The adiposity deposition in various tissues of the body is a matter of concern for the adolescents. Table III illustrates the effect of obesity among adolescents on advanced glycation end products. Obese adolescents have higher incidences of developing chronic metabolic conditions. The receptor for AGE (RAGE) is considered as one of the factors causing these complications. The linkage between AGE, RAGE and obesity is not well known, but few studies have shown an inverse relationship between them. *Norata et al*, demonstrated a similar finding in the adult population. In this study, BMI was considered as a predictive measure for sRAGE levels in terms of obesity indices. [25] Chih-Tsueng He *et al*, however observed a significant relationship between plasma RAGE and metabolic syndrome components, illustrating an increase in AGE leads to diabetic and cardiovascular consequences in the future. [26] Along with endogenous AGE, consumption of Dietary AGE has deleterious effects among adolescents causing an early carotid intima thickness and arterial stiffness. *Saha et al*, observed an increase in DAGE consumption among obese adolescents and a significant relationship between dAGE and metabolic compounds.[27]

*Rodríguez-Morteraa et al*, had a contrasting observation were, serum CML and Total AGE was decreased in obese adolescents but dAGE consumption was higher among them. [28] This can be attributed to the fact that fat mass or adipose tissue is inversely proportional to serum CML. This association could be explained by the deposition of CML in the adipose tissues regarded as hidden CML, thus non-reflecting the circulating total body CML. [29] Microinflammation as characterized by CRP levels were reported to be high among obese adolescents despite showing lower plasma CML levels. This is due to the famct that cytokine production such as the formation of IL-6, IL-1 is elevated in cases of increased adipocytes formation causing a rise in CRP levels.[30] Invitro studies done on animal model had shown the potential role of exogenous or diet derived AGE's inducing a microinflammation status in the rodents studied. [31] Oxidative stress is another compound which is elevated in obese individuals. Alongside, IL-6 and IL-1 there is another prominent predictor of oxidative stress which shares a similar characterization to advanced glycation end products. These are called as advanced oxidation protein products (AOPP). These protein oxidised compounds are increased during obesity and can be considered as an independent risk factor for metabolic syndrome in children or adolescents. [32] However, the role of triglycerides in exhibiting a significant correlation with AOPP has recently been reported. It has been studied, that oxidation of proteins is collateral with lipid peroxidation concluding an increase in TG levels during a high oxidative stress episode. [33]

Many studies have detected AGE through serum or plasma, but limited studies have focussed on non-invasive techniques in measuring AGE. One such technique involves the use of skin autofluorescence, where the deeper tissues of the skin have deposits of AGE which are detected under an AGE reader. [34] A study investigating SAF values on obese adolescents revealed a high skin AGE levels among obese subjects compared to lean controls. [35] it was also observed that duration of obesity plays an important role in SAF, i.e. morbid obesity showed high SAF values in comparison with short term obesity. Many contradicting factors arise involving skin autofluorescence. The skin type of the individuals i.e., Dark skin shows high AGE compared to lighter skin tones, appropriate technique to Measure SAF may vary, population of subjects- Asia, Hispanic, African American, White etc along with Age which also has a direct relationship with SAF. metabolic complications prominently cardiovascular disease and ESRD also play a significant role in higher SAF values among subjects. [36]

## CONCLUSION

This current review has highlighted upon the transitioning age group of adolescence and their exposure towards advanced glycation end products. The negative consequences such as development of cardiovascular and metabolic complications have been attributed solely due to an increase in dietary AGE consumption among the age group. Highly processed and high temperature cooking techniques have shown increases in Maillard reaction products leading to the formation of dietary AGE. Metabolic complications of AGE primarily focussed on diabetes mellitus and obesity which are commonly found among adolescents have been highlighted and show increases in HOMA-IR, LDL, Triglycerides, and anthropometric measures such as BMI and weight gain which have a direct relationship with glycation end products. Another interesting theory relates dimensional changes in the thyroid gland due to elevated AGE levels among adolescents. sRAGE (Receptor for AGE) after being attached to the AGE compound causes crosslinking of proteins which thus, was regarded as the main evil causing compound leading to metabolic diseases. Alongside, SRAGE there were other factors causing an increase in AGE, such as Age, BMI, Weight gain (Obesity), diagnostic procedures etc. Skin autofluorescence was a minimal non-invasivetechnique which had varying degrees of differentiation when analysed in darker skin tones and different races of the world making it an inaccurate diagnostic measure of AGE. Adolescents with a disease burden had increased exposure to AGE with an increase in CML levels compared to healthy controls. Hence, advanced glycation end products have negative consequences among the adolescent population and adequate steps should be taken to protect them for this glycated compound

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**Table I- The Impact Of Advanced Glycation End Products On Adolescents**

| Study | Study design | Sample size | Age group | Findings |
|---|---|---|---|---|
| Tommaso Aversa *et al*, 2021. | Case control study | 35 | <18 years | sRAGE levels decreased in the presence of euthyroid status of the individuals indicating the presence of oxidative damage in the subjects.[15] |
| Ma. Eugenia Garay-Sevilla *et al*, 2018. | Case control study | 160 | 12-19 years | This study showed the main findings of High serum CML and sRAGE in metabolically unhealthy obese adolescents compared to metabolically healthy obese.<br>A contrasting feature was observed in a normal weight adolescent presenting high serum CML and sRAGE levels similar to an obese adolescent.[17] |

**Table 2: Metabolic impact of Advanced Glycation End Product on Adolescents**

| Study | Study design | Sample size | Age group | Findings |
|---|---|---|---|---|
| Angela Galler, Grit Muller, Reinhard Schinzel *et al*, 2003. | Retrospective study | 159 | <20 years | Adolescents in the age of 13-16 years diagnosed with type-I DM had significantly higher levels of serum AGE's. In addition, lipid profile such as triglycerides and cholesterol showed higher incidence among the similar group in comparison with the healthy controls.[19] |
| Galler *et al*, 2003<br>Rezaei *et al*, 2022<br>Kostolanská *et al*, 2009 | Prospective study | 250 | <20 years | Hba1c and serum AGE have a direct relationship. High metabolic AGE levels and accelerates towards high hba1c levels. [20,21,22] |
| Francesco Chiarelli, MD, Maurizio de Martino *et al*, 1999. | Case-Control study | 178 | 6-21 years | This study reveals the presence of elevated serum AGE levels among adolescents in the age group of 6-12 years diagnosed with Type-I DM compared to healthy controls. This indicates the risk of microvascular complications among young adolescents at a faster rate. [23] |
| Stefano Zucchini, Marianna Fabi *et al*, 2020. | Cross-sectional study | 116 | 10-18 years | The study consisted of 2 groups. Group-I adolescents with T1DM and Group-II comprised of obese individuals.<br>Both the groups were evaluated for lipid profile, Anthropometric data, sAGE levels.<br>The results revealed high levels of sAGE among T1DM subjects compared to the obese category where, CV risk parameters seem to be elevated. Also, Gender and Age played a pivotal role in AGE per say. [24] |

**Table 3: Impact Of Obesity on Advanced Glycation End Products Among Adolescents**

| Study | Study design | Sample size | Age group | Findings |
|---|---|---|---|---|
| Chih-Tsueng He ,Chien-Hsing Lee, *et al*,2014 | Cross sectional study | 1083 | 12-16 years | Lipid profile, anthropometric parameters, BMI were inversely correlated with sRAGE Levels in males compared to females, where waist circumference & BMI were significantly correlated.<br>This study proves SRAGE levels are associated with metabolic syndrome and obesity. [26] |
| Saha *et al*, 2017 | Retrospective study | 46 million | 12-19 years | High dAGE consumption was observed among Metabolic Syndrome than Non-Metabolic Syndrome.<br>It was also observed that dietary AGE can be considered as an independent predictor for Metabolic syndrome. [27] |
| Reyna Rodríguez-Morteraa Claudia Luevano-Contreras, *et al*, | Cross sectional study | 66 | 15-19 years | Obese adolescents had elevated lipid parameters, Serum CML, Total AGE, DAGE intake, HOMA-IR and AI. This study proves a strong association between obesity and cardiometabolic risk, which in |

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|---|---|---|---|---|
| 2019. | | | | turn proves the presence of AGE endogenously and exogenously can have dire consequences. [28] |
| Katrien H.J. Gaens, *et al*, 2015. | Cohort study | 1270 | 40-60 years | Obesity had a direct relationship with inflammation evidenced by high CML and LGI Scores. [29] |
| Katari´Na Sˇ Ebekova , Veronika Somoza, *et al*, 2009. | Observational study | 18 obese 18 non-obese | 5-18 years 4-17 years | Oxidative stress i.e CRP, IL-6 along with HOMA-IR showed increased levels in obese subjects compared to lean controls. Whereas, serum CML levels were lower in obese adolescents.[30] |
| Melpomeni Peppa *et al*, 2003. | Experimental study | 105 | Diabetic Rats | High AGE diet mice had delayed wound healing (epithelialization) compared to low AGE diet indicating a direct role of glycation causing inflammation and skin AGE deposition. [31] |
| Malgorzata Krzystek-Korpacka a, Eliza Patryn, *et al*, 2008. | Cross sectional study | 114 obese 53 healthy controls | <17 years old | AOPP (Advanced oxidation protein products) were elevated in obese subjects compared to lean controls. Lipid parameters, Insulin and central obesity were positively correlated with AOPP indicating AOPP as a risk factor for the development of obesity. [32] |
| Korkmaz, G. G *et al*, 2013. | Cross sectional study | 55 MetS pts 20 Healthy controls | 40-50 years | AOPP values were higher and directly proportional to waist circumference (Obesity) in MetS patients due to the presence of oxidative stress causing free radical damage and inflammation in the body. [33] |
| Xian Wang, *et al*, 2021 | Cross sectional study | 825 | 18-80 years | Skin autofluorescence considered as a predictor for diabetic complications especially diabetic retinopathy, neuropathy, cardiovascular and kidney disease in patients with diabetes mellitus. [34] |
| Yvette E. Lentferink, Lisa van Teeseling, *et al*, 2018. | Cross sectional study | 571 | 4-18 years | SAF (Skin Auto fluorescence) was measured in the adolescent subjects with obesity and healthy controls. High levels of SAF were observed in obese subjects revealing the linkage between obesity and high AGE levels in all age groups except <10years. [35] |
| Cidila Da Moura *et al*, 2017. | Review article | Nil | Nil | SAF can be considered as a biomarker to predict diabetes complications due to "long term memory" compared to Hba1c (3 months average) [36] |
| S. Accacha a W. Rosenfeld, *et al*, 2013. | Cross sectional study | 88 | 11-15 years | CML levels were below than the average adult values. Also, CML levels had a negative correlation with adiposity elucidating AGE as not a complete risk factor for adiposity related comorbidities. [37] |
| Mohamed Rowisha, Manal El-Batch, *et al*, 2016. | Cross sectional study | 50 obese 40 healthy controls | >18 years old | Obese group had increased cIMT, Lipid parameters and Insulin resistance. Increased expression of AGE, ADMA was also observed. sRAGE was decreased in obese adolescents compared to controls. and alsosRAGE correlated with BMI stating body mass as a good predictor for AGE levels. [38] |

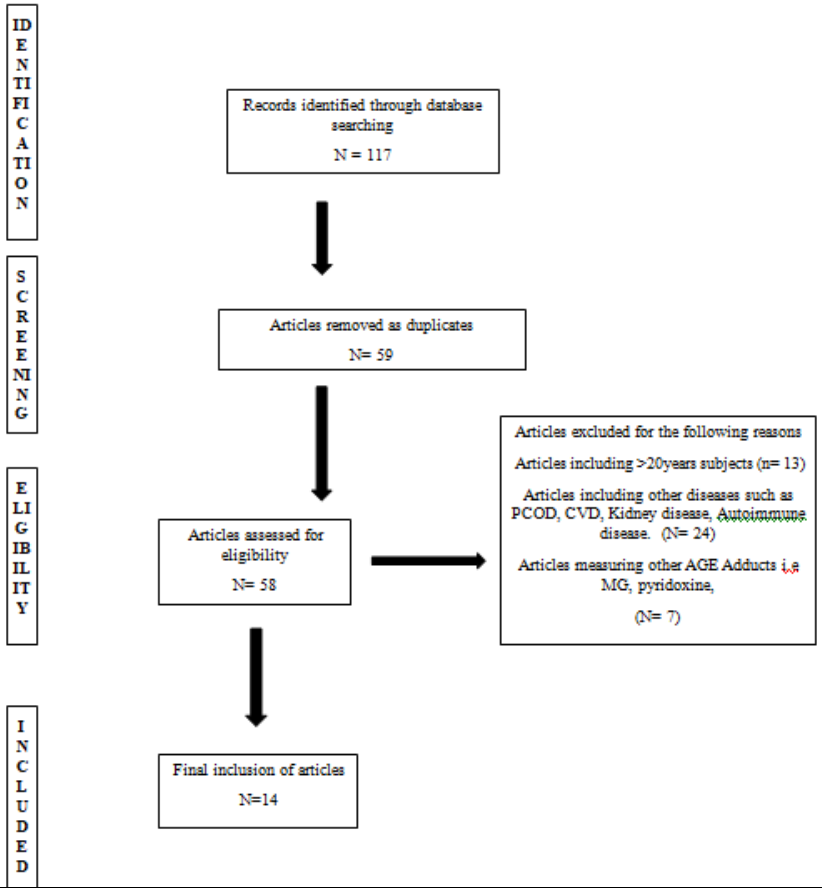


**Figure I: A Schematic representation of the systematic process of selection of articles.**

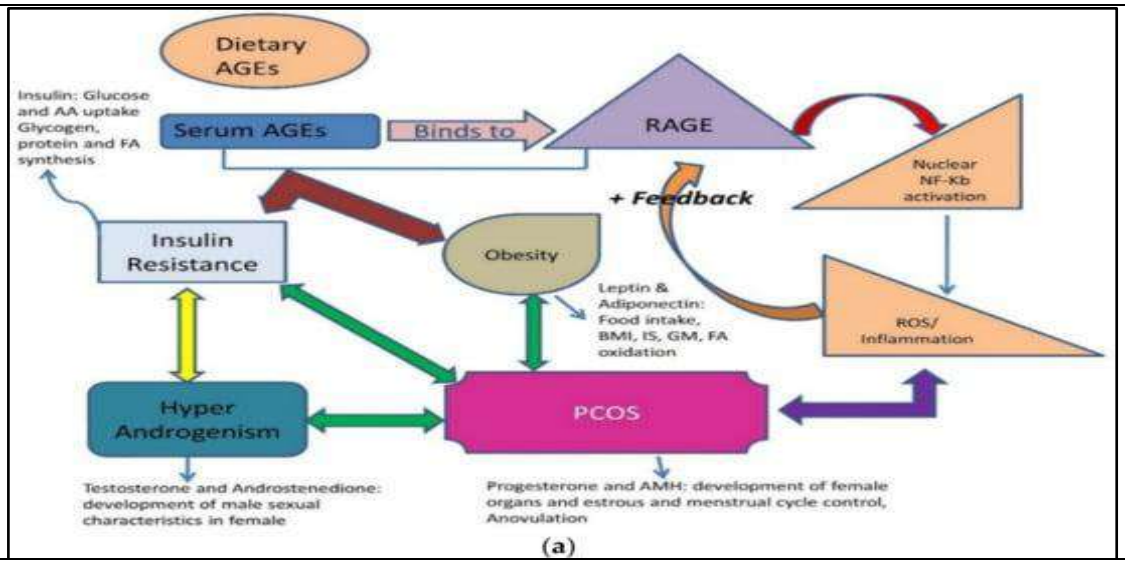


**Figure II: Dietary AGE's leading to the development of metabolic syndrome, *Gill et al, 2019*.[18]**

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**RESEARCH ARTICLE**

# Lab Scale Study of Catalytic Degradation Kinetics for Textile Dye : Tetrazine

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**ABSTRACT**

This study explores the degradation kinetics of the textile dye Tetrazine using photocatalysis. Tetrazine, prevalent in the textile industry, is environmentally persistent and potentially toxic. The research aims to understand its degradation mechanisms and rate constants under various conditions, including pH, temperature, and the presence of catalysts like UV light, air, titanium dioxide ($TiO_2$), and hydrogen peroxide ($H_2O_2$). Controlled experiments monitored Tetrazine and its degradation products using high-performance liquid chromatography (HPLC) and UV-Vis spectroscopy. Results show that Tetrazine degradation follows first-order kinetics, with the rate influenced by pH and temperature. Acidic conditions and higher temperatures accelerate the degradation process. Notably, $TiO_2$ photocatalysis under UV light and the addition of $H_2O_2$ significantly enhance the degradation rate. These findings contribute to effective strategies for treating dye-contaminated wastewater, emphasizing the role of optimized conditions and advanced photocatalytic techniques. Further research is suggested to fully understand degradation pathways and ensure environmental safety by identifying non-toxic byproducts.

**Keywords:** Textile Dye, Tetrazine, $TiO_2$, Pollution, degradation

## INTRODUCTION

The textile industry is one of the largest consumers of synthetic dyes, which are widely used to impart colour to fabrics. Among these dyes, Tetrazine is notable for its vivid colours and durability. However, the extensive use of Tetrazine poses significant environmental and health risks due to its persistence in the environment and potential toxicity (Babu, Parande, Raghu, & Kumar, 2007). The discharge of dye-laden wastewater into natural water bodies leads to severe water pollution, affecting aquatic life and human health (Saratale et al., 2011). Degradation of synthetic dyes like Tetrazine in wastewater is challenging due to their complex molecular structures and resistance to biodegradation (Robinson, McMullan, Marchant, & Nigam, 2001). Traditional wastewater treatment methods often

fail to completely remove these dyes, necessitating the development of more effective degradation techniques (Forgacs, Cserháti, & Oros, 2004). Among the advanced treatment methods, photocatalysis has emerged as a promising approach due to its efficiency in degrading a wide range of organic pollutants (Chong, Jin, Chow, & Saint, 2010). Photocatalysis involves the use of a catalyst, typically titanium dioxide ($TiO_2$), under UV light to generate reactive oxygen species that can break down complex organic molecules (Hoffmann, Martin, Choi, &Bahnemann, 1995). Studies have shown that the combination of $TiO_2$ and UV light significantly enhances the degradation of various dyes (Daneshvar, Salari, &Khataee, 2004). Additionally, the presence of oxidizing agents like hydrogen peroxide ($H_2O_2$) can further accelerate the degradation process by generating additional reactive species (Pera-Titus, García-Molina, Baños, Giménez, &Esplugas, 2004).

This study aims to investigate the degradation kinetics of Tetrazine using photocatalysis under different environmental conditions. By examining the effects of pH, temperature, UV light, $TiO_2$, air, and $H_2O_2$, this research seeks to provide a comprehensive understanding of the factors influencing Tetrazine degradation. The findings will contribute to the development of effective treatment strategies for dye-contaminated wastewater, helping to mitigate the environmental impact of textile dyes.

## MATERIALS AND METHODS

## MATERIALS

- **Tetrazine Dye**: Analytical grade Tetrazine dye in solid, orange coloured was procured from Sigma-Aldrich.(Fig-1)
- **Titanium (iv) Dioxide ($TiO_2$)**: It is also known as rutile or anatase consist of mixture of two forms. P25 grade $TiO_2$, with an average particle size of 21 nm and a specific surface area of 50 $m^2/g$, was used as the photocatalyst. Purity was 99.9 % and used without further purifications.
- **Hydrogen Peroxide ($H_2O_2$)**: 30% (w/v) hydrogen peroxide solution was obtained from Merck. Used without further purifications.
- **Other Chemicals**: Sodium hydroxide (NaOH) and hydrochloric acid (HCl) for pH adjustment were sourced from Sigma-Aldrich.
- **Deionized Water**: All solutions were prepared using deionized water.

## METHODS

The method provided a comprehensive approach to study the degradation kinetics of Tetrazine under various conditions. The setup for these experiments mirrored that of the primary photocatalytic tests, ensuring that any degradation observed was due to non-photocatalytic processes. This includes potential adsorption onto the catalyst surface or chemical reactions that do not depend on light.

**Experimental Setup**

1. **Photocatalytic Reactor**: A batch photocatalytic reactor equipped with a UV lamp ($\lambda$ = 365 nm) was used for the degradation experiments. The reactor was designed to maintain a constant temperature and ensure uniform UV light exposure.
2. **Preparation of Dye Solution**: A stock solution of Tetrazine dye (100 mg/L) was prepared by dissolving the appropriate amount of dye in deionized water. Experimental solutions were obtained by diluting the stock solution to the desired concentrations.
3. **Catalyst Preparation**: $TiO_2$ was dispersed in deionized water to achieve a final concentration of 1 g/L. The suspension was sonicated for 30 minutes to ensure uniform dispersion.

**Experimental Procedure:**

1. **Effect of pH**: The pH of the dye solution was adjusted to the desired levels (pH 3, 7, and 11) using NaOH and HCl. The solution (100 mL) was then placed in the photocatalytic reactor with $TiO_2$ (1 g/L) and exposed to UV light. Samples were taken at regular intervals for analysis.
2. **Effect of Temperature**: Experiments were conducted at different temperatures (25°C, 35°C, and 45°C). The temperature was controlled using a water bath. The dye solution (100 mL) with $TiO_2$ (1 g/L) was exposed to UV light, and samples were collected periodically.
3. **Effect of Hydrogen Peroxide**: To investigate the role of $H_2O_2$, different concentrations (0.1%, 0.5%, and 1% w/v) were added to the dye solution (100 mL) with $TiO_2$ (1 g/L). The mixture was exposed to UV light, and samples were taken at specific time intervals.
4. **Sample Analysis**: The concentration of Tetrazine in the samples was measured using high-performance liquid chromatography (HPLC) equipped with a UV detector. The mobile phase consisted of acetonitrile and water (70:30 v/v), and the detection wavelength was set at 254 nm. Additionally, UV-Vis spectroscopy was used to monitor the absorbance changes at the dye's maximum absorption wavelength ($\lambda$_max = 540 nm).
5. **Kinetic Analysis**: The degradation kinetics were analyzed by plotting the natural logarithm of the Tetrazine concentration against time. The rate constants were determined from the slopes of the linear regression lines, assuming first-order kinetics.

**Control Experiments**

- **Dark Control**: Experiments were conducted in the absence of UV light to assess the contribution of photocatalysis.
- **Catalyst Control**: Experiments without $TiO_2$ were performed to evaluate the effect of UV light alone.
- **Oxidant Control**: Experiments without $H_2O_2$ were conducted to assess the combined effect of $TiO_2$ and UV light without the oxidizing agent.

**Data Analysis**

In order to get better results with perfection, all experiments were performed in triplicate, and the average values were reported. The standard deviation was calculated to determine the reproducibility of the results. This methodological approach ensures a comprehensive understanding of the degradation kinetics of Tetrazine and the influence of various parameters on the photocatalytic process.

## RESULTS AND DISCUSSION

Below is a tabulated representation of the data collected from the various experimental conditions examined in the study. The data includes the concentration of Tetrazine dye over time, measured under different conditions of pH, temperature, and the presence of catalysts ($TiO_2$ and $H_2O_2$). This tabulation data provides a clear overview of how various factors such as pH, temperature, and the presence of catalysts influence the degradation of Tetrazine dye. Each table highlights the effect of specific conditions, facilitating a comprehensive analysis of the degradation kinetics. The data presented in the tables provide insights into the degradation kinetics of Tetrazine dye under various experimental conditions, including the effects of pH, temperature, and the presence of catalysts such as $TiO_2$ and $H_2O_2$. This discussion interprets the observed trends and elucidates the underlying mechanisms of Tetrazine degradation.

**Effect of pH on Tetrazine Degradation**

Table 1 shows the concentration of Tetrazine over time at different pH levels (3, 7, and 11). The degradation rate is significantly higher at acidic pH (3) compared to neutral (7) and alkaline (11) conditions. At pH 3, the concentration of Tetrazine decreases from 10.0 mg/L to 1.1 mg/L in 60 minutes, indicating rapid degradation. In contrast, at pH 7, the concentration decreases to 3.0 mg/L, and at pH 11, it decreases to 6.3 mg/L over the same period.(Fig,-2)

The enhanced degradation at acidic pH can be attributed to the increased generation of hydroxyl radicals (•OH) in acidic conditions, which are highly reactive and effective in breaking down organic dye molecules (Chen, Mao, & Zhao, 2012). The lower degradation rates at neutral and alkaline pH suggest that fewer hydroxyl radicals are generated, leading to slower degradation.

**Effect of Temperature on Tetrazine Degradation**

Table 2 illustrates the effect of temperature on Tetrazine degradation at pH 7. As the temperature increases from 25°C to 45°C, the degradation rate significantly increases. At 25°C, the concentration of Tetrazine decreases from 10.0 mg/L to 3.0 mg/L in 60 minutes. At 35°C, it drops to 1.2 mg/L, and at 45°C, it reaches as low as 0.2 mg/L. (Fig-3). The accelerated degradation at higher temperatures can be explained by the increased kinetic energy of the molecules, which enhances the reaction rates. Higher temperatures also improve the generation of reactive species such as hydroxyl radicals during the photocatalytic process (Gaya & Abdullah, 2008). This result is consistent with the Arrhenius equation, which states that the reaction rate increases exponentially with temperature.

**Effect of Oxidant Hydrogen Peroxide on Tetrazine Degradation**

Table 3 highlights the impact of adding hydrogen peroxide ($H_2O_2$) to the dye solution with $TiO_2$ under UV light at pH 7 and 25°C. The degradation rate increases with higher concentrations of $H_2O_2$. Without $H_2O_2$, the concentration of Tetrazine decreases to 6.5 mg/L in 60 minutes. With 0.1% $H_2O_2$, it decreases to 1.0 mg/L, with 0.5% $H_2O_2$, it decreases to 0.2 mg/L, and with 1% $H_2O_2$, it almost completely degrades to 0.1 mg/L. (Fig-4). Hydrogen peroxide acts as an additional source of hydroxyl radicals when exposed to UV light, further enhancing the photocatalytic degradation process (Pera-Titus et al., 2004). The significant increase in degradation rate with higher $H_2O_2$ concentrations demonstrates its effectiveness in accelerating the breakdown of Tetrazine. (Fig,-5)

**Control Experiments**

Table 4 presents the results of control experiments conducted in the absence of UV light (dark control), $TiO_2$, and $H_2O_2$. The degradation rates in these control conditions are significantly lower compared to the experimental conditions with UV light, $TiO_2$, and $H_2O_2$. In the dark control, the concentration of Tetrazine decreases only slightly from 10.0 mg/L to 8.8 mg/L in 60 minutes, indicating minimal degradation. Similarly, without $TiO_2$ and $H_2O_2$, the concentrations decrease to 8.7 mg/L and 6.5 mg/L, respectively. The graphical representations of all three control experiments are incorporated with all charts above. These control experiments confirm that the presence of UV light, $TiO_2$, and $H_2O_2$ is crucial for effective degradation of Tetrazine. The minimal degradation in the absence of these factors highlights the importance of photocatalysis and the synergistic effect of the catalysts in enhancing the degradation process (Hoffmann et al., 1995).

## CONCLUSION

The discussion of the tabulated data demonstrates that the degradation of Tetrazine dye is significantly influenced by pH, temperature, and the presence of catalysts such as $TiO_2$ and $H_2O_2$. Acidic conditions, higher temperatures, and the addition of hydrogen peroxide enhance the degradation rate, supporting the use of optimized photocatalytic processes for effective treatment of dye-contaminated wastewater. These findings contribute to the development of efficient strategies for mitigating the environmental impact of textile dyes. Further research should focus on understanding the degradation pathways and identifying non-toxic by products to ensure comprehensive environmental safety.

## ACKNOWLEDGEMENT

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**Table 1: Effect of pH on Tetrazine Degradation with $TiO_2$ under UV Light**

| Time (min) | Concentration (mg/L) at pH 3 | Concentration (mg/L) at pH 7 | Concentration (mg/L) at pH 11 |
|---|---|---|---|
| 0 | 10.0 | 10.0 | 10.0 |
| 10 | 8.2 | 8.9 | 9.5 |
| 20 | 6.5 | 7.8 | 9.0 |
| 30 | 4.7 | 6.7 | 8.3 |
| 40 | 3.2 | 5.4 | 7.6 |
| 50 | 2.0 | 4.2 | 7.0 |
| 60 | 1.1 | 3.0 | 6.3 |

**Table 2: Effect of Temperature on Tetrazine Degradation with $TiO_2$ under UV Light at pH 7**

| Time (min) | Concentration (mg/L) at 25°C | Concentration (mg/L) at 35°C | Concentration (mg/L) at 45°C |
|---|---|---|---|
| **0** | **10.0** | **10.0** | **10.0** |
| **10** | **8.9** | **7.5** | **6.3** |
| **20** | **7.8** | **5.6** | **3.9** |
| **30** | **6.7** | **4.1** | **2.0** |
| **40** | **5.4** | **3.0** | **1.1** |
| **50** | **4.2** | **2.0** | **0.6** |
| **60** | **3.0** | **1.2** | **0.2** |

**Table 3: Effect of Hydrogen Peroxide on Tetrazine Degradation with $TiO_2$ under UV Light at pH 7 and 25°C**

| Time (min) | Concentration (mg/L) with 0.1% $H_2O_2$ | Concentration (mg/L) with 0.5% $H_2O_2$ | Concentration (mg/L) with 1% $H_2O_2$ |
|---|---|---|---|
| 0 | 10.0 | 10.0 | 10.0 |
| 10 | 6.8 | 5.2 | 4.0 |

| | | | |
|---|---|---|---|
| 20 | 4.7 | 2.8 | 1.8 |
| 30 | 3.5 | 1.6 | 0.8 |
| 40 | 2.4 | 0.9 | 0.4 |
| 50 | 1.6 | 0.5 | 0.2 |
| 60 | 1.0 | 0.2 | 0.1 |

**Table 4: Control Experiments**

| Time (min) | Concentration (mg/L) in Dark<br>Dark Control | Concentration (mg/L) With UV only<br>Catalyst Control | Concentration (mg/L) With UV + $TiO_2$<br>Oxidant Control |
|---|---|---|---|
| 0 | 10.0 | 10.0 | 10.0 |
| 10 | 9.8 | 9.7 | 9.3 |
| 20 | 9.6 | 9.5 | 8.7 |
| 30 | 9.4 | 9.3 | 8.2 |
| 40 | 9.2 | 9.1 | 7.6 |
| 50 | 9.0 | 8.9 | 7.1 |
| 60 | 8.8 | 8.7 | 6.5 |

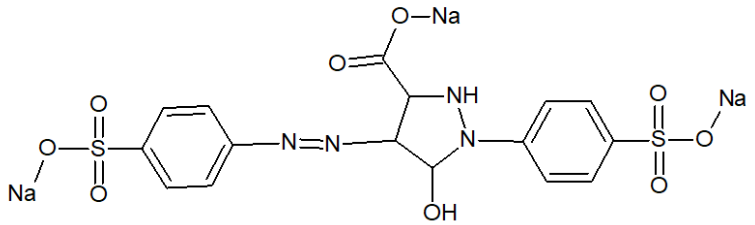


**Figure 1: Structure of tetrazine salt**

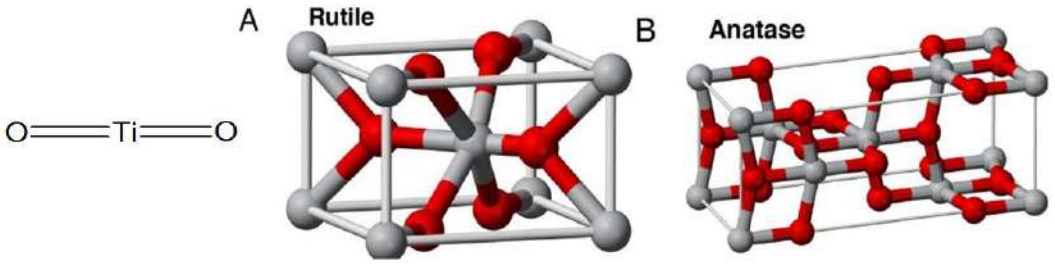


**Figure 1: Structure of $TiO_2$(Austin, R. H., & Lim, S. F. 2008).**

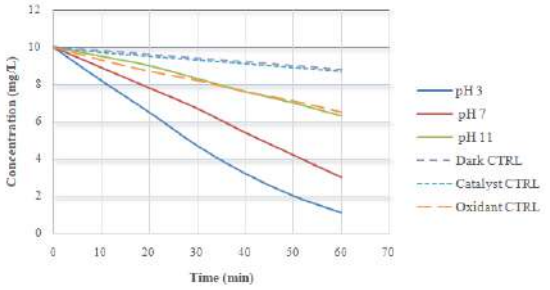


**Figure 2: Effect of pH on Tetrazine Degradation with $TiO_2$ under UV Light**

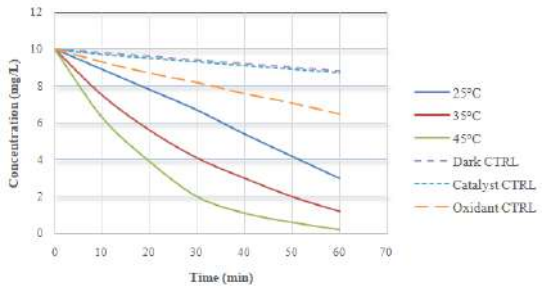


**Figure 3: Effect of Temperature on Tetrazine Degradation with $TiO_2$ under UV Light at pH 7**

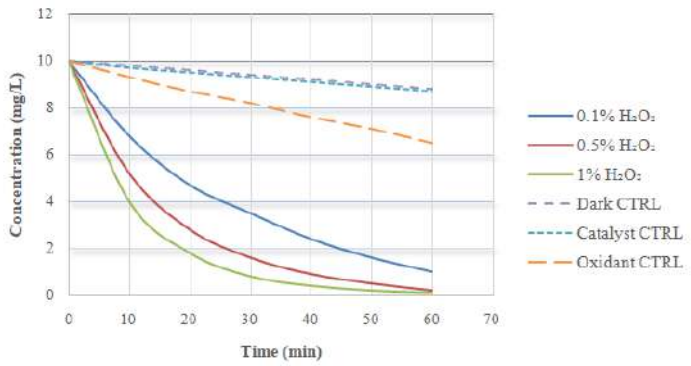


**Figure 4: Effect of $H_2O_2$ on Tetrazine Degradation with $TiO_2$ under UV Light at pH 7 and 25°C**

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**RESEARCH ARTICLE**

# A Numerical Study on the Flow of Oil-based Fuzzy Hybrid Nanomaterial Stretching Cylinder by Modified Buongiorno's Model

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## ABSTRACT

A model of Carreau fluid flow is estimated with the velocity and energy transmission over the hybrid nanofluid (hnf) past a stretching cylinder by modified Buongiorno's model. By combining nanoparticles with distinct rheological and thermal characteristics, the nanofluids proficiency might be increased, stabilized, and more effective. The dispersion has created a hybrid nanofluid of Manganese-zinc ferrite($\boldsymbol{MnZnFe_2O_4}$) and Nickel-zinc ferrite ($\boldsymbol{NiZnFe_2O_4}$) nanocomposites in the base fluid. To assess the permeability effect, the momentum equation incorporates the consequences of the Weissenberg number. The flow of fluid is controlled by a system of PDEs that take into account the consequences of a heat source, and natural convection. They are then transformed, using substitutions for similarity variables, into a non-dimensional set of ODEs. In the second section, we discussed hnf and nanofluid, moment, and energy through the fuzzy context. The volume quantity of hnf in $\boldsymbol{MnZnFe_2O_4}$ and $\boldsymbol{NiZnFe_2O_4}$ is considered unknown concerning triangular fuzzy numbers (TFNs) [0, 0.05, 0.1]. The triangular membership function (TMF) is utilized to research variability and uncertainty, while $\alpha -$ cut controls the TFNs.The velocity field of reaction in hybrid nanofluid ($\boldsymbol{MnZnFe_2O_4 + NiZnFe_2O_4/C_nH_{2n+2}}$)and nanofluid ($\boldsymbol{MnZnFe_2O_4/C_nH_{2n+2}}$) developed by Weissenberg number and curvature parameter, the heat conduction of reaction in hnf and nanofluid developed by Prandtl number. When evaluating the fields of velocity, temperature, coefficient of skin friction, and local Nusselt numeral, the results are displayed in tables and graphs.

**Keywords:** Carreau hnf, Cylinder, Modified Buongiorno's method, TFNs, TMF, bvp5c.

# INTRODUCTION

The present section discusses the numerous uses and applications of hybrid nanofluid ($MnZnFe_2O_4 + NiZnFe_2O_4 + C_nH_{2n+2}$) associated with nanofluid ($MnZnFe_2O_4 + C_nH_{2n+2}$) some samples in this field incorporate fuel manufacturing, advanced frosty techniques, contemporary medicinal conveyance, apparatus, nanofabrication, transmission of energy, and cooling system in electronic circuits. As a result of this significant achievement, substantial research has been undertaken globally on energy transfer and flow properties.Hybrid nanofluids are a new nanotechnological type that is frequently formed by spreading two distinct kinds of nanoparticles in base fluids. Several researchers have applied hybrid nanofluids. Khashi et al. [1] examined magneto hydrodynamics hybrid nanofluid in water flow on a moveable surface using the Joule thermal consequence. Nazir et al. [2] analyzed the modifications in Joule heating, magnetic induction, and chemical response composition that have significant effects on flow and heat transfer during species transport. Dogonchi et al. [3] studied the hybrid nanostructures in fluid spinning as a result of surface rotation and they applied the Duan-Rach method to the predicted problem and observed an increase in the heat proficiency of the fluid caused by hnf. Kumar et al. [4] examined the numerical study completed to explore the analysis of heat conduction and flow of anhnf flow above a porous material. Ghadikolaei [5] demonstrated the 3D flow and heat conduction of $TiO_2 - CuO$/hnf floating in the base fluid. Dogonchi et al. [6] studied the finite element approach for control volume analysis$Fe_3O_4 - H_2O$ nanofluid in an annulus region subjected to thermal transmission. Waqaset al. [7] the organization of hybrid nanomaterial flow through porous media have been examined and the consequence of the magnetic fields and nanofluid radiation are significant in biotechnology and medicine. Reddy et al. [8] illustrated the flow of anhnf $Al_2O_3 - cu$/Ethyleneglycol across a narrow, moving tip using magnetohydrodynamic, Dufour-Soret consequence, and infrared radiation. Kavya et al. [9] investigated the Williamson hnf in a stretched cylinder containing based on the water$Cu/MoS_4$ nanoparticles. The heat transmission capabilities of the Williamson hnf flow decrease as Prandtl numeral and curvature limiting factor values increase. Sulochana et al. [10] covered the investigation of the magneto hydro dynamic nf flow perimeter film, as well as arranged symmetrically, constantly shifting the needle. Madhukesh et al. [11] exploredthe idea of a liquid hybrid nanomaterial passing through a tiny needle with the help of magnetic flow, thermophoresis effect, and convection boundary conditions. Salleh et al. [12] examined while accounting for buoyancy effects, wall heat transmission, and an incompressible flow on a thin, fragile needle saturated with a$AI_2O_3 - Cu/water$ flow. Buongiorno [13] using mathematical nanofluid models, statistically examined the convection of the movement of a nanofluid in cavities with porous channels and viscous fluid.

In addition to chemical diffusion, there is an increase in an extensive range of concerns, including heat transmission. A fundamental role for fluid flow is played in engineering and science. After regulating, these models of physical problems are transmuted into non-linear differential equations. Using physical replication, which includes coefficients, 3D/2Dgeometry, limiting factors, circumstances, and boundary states, frequently has a significant influence on the alternatives of differential equations. Consequently, the limiting factors, initial and boundary specifications are virtually imprecise and caused by experimental error, coefficients, mechanical flaws, and dimension inaccuracy. Consequently, in this instance, methodology is a more accurate means of describing the phenomenon under investigation than presuming the presence of distinct or widespread physical problems. Fuzzy differential equations (FDEs) are particularly effective. Kaleva[14]introduced the concept of FDEs (fuzzy differential equations). Siddique et al. [15] described the fuzzy evaluation for mono-directional magneto hydrodynamics flow and examined ternary key flow issues involving a 3rd-grade liquid between two equal plates. Dubois et al. [16] developed mathematical methods for three distinct varieties of fuzzy numbers, triangular, trapezoidal, and Gaussian fuzzy numbers. For the sake of completeness, TFNs are listed. Seikkala [17] acquainted with the notion of fuzzy differentiation. Almutairi et al. [18] obtained the fuzzy waves equation numerical solution. Salahsour et al. [19] examined the alley effect using fuzzy differential equations and the fuzzy logistic equation with the assistance of TFNs. Merkin [20] examined flow reactions at the border layer when there was a homogenous or heterogeneous shortage of reactants. They discovered that the external response is a highly effective method for differentiating the

edge because the homogenous reaction occurs when the boundary layer is generated with decreased reactant surface concentration.

A stretching cylinder with an exponential curvature is affected by Buongiorno's Carreau fuzzy hybrid nanofluid model, although more research is required, according to the literature review. Mixing Maganese-Zinc ferrite ($MnZnFe_2O_4$) and Nickel-Zinc ferrite ($NiZnFe_2O_4$) nanoparticles are combined to create the hnf. A system of PDE was used to model the nanofluid flow; these associations were subsequently transformed into an ODE set based on similarities. The resultant system of equations pertaining to the boundary value problem was solved using the bvp5capproach. The following factors are estimated and evaluated: coefficient of skin friction, local Nusselt numeral, velocity, and temperature. The present investigation is novel in the subsequent aspects:

- Comparative study to ascertain the hybrid nanoparticles ($MnZnFe_2O_4$and $NiZnFe_2O_4$) utilizing oil as a base fluid.
- The behavior of hybrid nanofluids (hnf) is represented by a curved stretched cylinder. Apart from velocity, temperature, coefficient of skin friction,and local Nusselt numeral was also analyzed.
- The volume fraction of the epistemic uncertainty dispersion nanoparticles is calculated using fuzzy differential equations. Thus, the volume proportions of the hnf are regarded as TFNsalong with the use of the $\alpha$ −cut method. The$\alpha$ −cut is explained by fuzzy TMF.

Basics of Fuzzification [22]

This section presents a comprehensive explanation of fuzzy characteristics that can be used for subsequent calculations.

Definition 1.A set of fuzzy numbers is characterized as an ordered pair set given that

$\bar{\mathbf{A}} = \{(\boldsymbol{\eta}, \boldsymbol{\mu}_{\bar{\mathbf{A}}}(\boldsymbol{\eta})) : \mathbf{f} \in \mathbf{X}, \boldsymbol{\mu}_{\bar{\mathbf{A}}}(\boldsymbol{\eta}) \in [\mathbf{0}, \mathbf{1}]\}$. Where $\boldsymbol{\mu}_{\bar{\mathbf{A}}}(\mathbf{f})$ is a membership function of $\bar{\mathbf{A}}$, $\mathbf{X}$–all-encompassingset, and the mapping is measured as $\boldsymbol{\mu}_{\bar{\mathbf{A}}}(\boldsymbol{\eta}) : \mathbf{X} \longrightarrow [\mathbf{0}, \mathbf{1}]$.

Definition 2.$\alpha$ −Cut of a fuzzy set $\bar{A}$ is a crisp set $A_\alpha$ is explicated$A_\alpha = \{\eta / \mu_{\bar{A}}(\eta) \geq \alpha\}$, where$\alpha \in [0,1]$.

Definition 3.Let triangular fuzzy number (TFN) $\bar{A} = (b_1, b_2, b_3)$ along with membership function $\mu_{\bar{A}}(\eta)$explicate as,

$$\mu_{\bar{A}}(\eta) = \begin{cases} \frac{\eta - b_1}{b_2 - b_1}, \text{for } b_1 \leq \eta \leq b_2 \\ \frac{b_3 - \eta}{b_3 - b_2}, \text{for } b_2 \leq \eta \leq b_3 \\ 0, \quad \text{otherwise} \end{cases} \tag{1}$$

The $\alpha$-cut technique is used to transform a TFN with an upper limit (or center) value of $b_2$into interval numbers, where $\alpha$ is an integer between 0 and 1. Then, the formula $\bar{A} = [f_1(\eta;\alpha), f_2(\eta;\alpha)] = [b_1 + (b_2 - b_1)\alpha,\ b_3 - (b_3 - b_2)\alpha]$, is used to represent this transformation. The membership function defines the membership function, which is an essential part of fuzzy set theory. The $\alpha$-cut membership function and triangular fuzzy numbers A $\bar{A} = (b_1, b_2, b_3)$ are shown in Fig 1. In the following conditions, a triangular fuzzy number can be anything: On the interval [0, 1], the function $f_1(\eta;\alpha)$ rises and function$f_2(\eta;\alpha)$decreasing. $f_1(\eta;\alpha) \leq f_2(\eta;\alpha)$ on the interval [0,1]. The set [0,1] contain the bounds of $f_1(\eta;\alpha)$, and$f_2(\eta;\alpha)$. This means that$f_1(\eta,\alpha) = f_2(\eta,\alpha) = f(\eta)$, here$f(\eta)$ is crisp velocity.

## Mathematical formulation

A Carreau hybrid nanofluid flow in an incompressible manner through Buongiorno's model elongating cylinder exhibits both homogeneous and heterogeneous reactions for the cylinder. The physical attributes of the hnf are described in Table 1developed by Ahmad et al. [21] are compatible. Simplified homogeneous-heterogeneous responses can be expressed as follows, using the previously described presumptions and Merkin's theoretical [20] concepts.

$A + 2B \rightarrow 3B$, rate $= d_c ab^2$ (2)

One first-order isothermal reaction is taking place on the surface of the catalyst.

$A \rightarrow B$, rate $= d_s a$ (3)

The symbols a and b depict the concentrations of chemical categories A and B,$d_c$ and $d_s$ are the constants of rate.The model regulating equations are created after applying common boundary layer approximations,

$$\frac{\partial}{\partial x}(ru) + \frac{\partial}{\partial r}(rv) = 0, \quad (4)$$

$$u\frac{\partial u}{\partial x} + v\frac{\partial u}{\partial r} = \frac{\mu_{hnf}}{\rho_{hnf}}\frac{1}{r}\frac{\partial}{\partial r}\left[\left(r\frac{\partial u}{\partial r}\right)\left(1+\Gamma^2\left(\frac{\partial u}{\partial r}\right)^2\right)\right]^{\frac{n-1}{2}} + \frac{\mu_{hnf}}{\rho_{hnf}}(n-1)\Gamma^2\frac{\partial^2 u}{\partial r^2}\left(\frac{\partial u}{\partial r}\right)^2\left[1+\Gamma^2\left(\frac{\partial u}{\partial r}\right)^2\right]^{\frac{n-3}{2}}, \quad (5)$$

$$u\frac{\partial T}{\partial x} + v\frac{\partial T}{\partial r} = \frac{k_{hnf}}{r(\rho C_p)_{hnf}}\frac{\partial}{\partial r}\left[r\frac{\partial T}{\partial r}\right] + \tau\left[D_B\frac{\partial b}{\partial r}\frac{\partial T}{\partial r} + \frac{D_T}{T_\infty}\left(\frac{\partial T}{\partial r}\right)^2\right], \quad (6)$$

$$u\frac{\partial a}{\partial x} + v\frac{\partial a}{\partial r} = D_A\left[\frac{\partial^2 a}{\partial r^2} + \frac{\partial a}{\partial r}\right] + D_A\frac{\partial a}{\partial r} + \frac{D_T}{T_\infty}\frac{\partial T}{\partial r} - d_c ab^2, \quad (7)$$

$$u\frac{\partial b}{\partial x} + v\frac{\partial b}{\partial r} = D_B\left[\frac{\partial^2 b}{\partial r^2} + \frac{\partial b}{\partial r}\right] + D_B\frac{\partial b}{\partial r} + \frac{D_T}{T_\infty}\frac{\partial T}{\partial r} + d_s ab^2. \quad (8)$$

For Equations (3)– (7), the boundary conditions are stated as follows

$$\boldsymbol{u = u(x), \quad v = 0, D_A\frac{\partial a}{\partial r} = d_c a, \ D_B\frac{\partial b}{\partial r} = -d_s a, \quad T = T_w, \text{ at } r = R,}$$

$$u \rightarrow 0, \ T \rightarrow T_\infty, \ a \rightarrow a_\infty, \ b \rightarrow b_\infty \ \ as \ \ r \rightarrow \infty. \quad (9)$$

A and B are the concentrations of hnf particles, $u(x) = \frac{xu_0}{l}$ where $u_0$ velocity,$x$ is an independent ,$l$ is the characteristic length, $a$ and $b$ are concentrations of nanoparticles$[mol/m^3]$,$D_A$ and $D_B$ are the Brownian diffusion coefficients$[m^2/s]$, $D_T$ is the thermophoresis diffusion coefficient$[m^2/s]$, u and v are velocity components $[m/s]$, $(C_p)_{hnf}$ is the specific heat of hnf $[J/kg.K]$,$\mu_{hnf}$is the dynamic viscosity of the hnf$[kgm^{-1}s^{-1}]$, $\rho_{hnf}$is the density of hnf $[kg/m^3]$, $(\rho C_p)_{hnf}$ is the heat capacitance of hnf $[J/kg.K]$, $\nu_{hnf}$is the kinematic viscosityof the hnf$[m^2/s]$,$k_{hnf}$ is the thermal conductivity of hnf $[W/m.K]$, $\alpha_{hnf}$is the thermal diffusivity of the hnf $[m^2/s]$,$n$is the power law index number, and the effective fluid heat capacity $\tau = \frac{(\rho C)_f}{(\rho C)_p}$.

$D_A\frac{\partial a}{\partial r} + \frac{D_T}{T_\infty}\frac{\partial T}{\partial r} = 0$ and $D_B\frac{\partial b}{\partial r} + \frac{D_T}{T_\infty} = 0$claimthat when thermophoresis is considered, the typical flow of hnf particles at the interface is zero.

$$\frac{\rho_{hnf}}{\rho_f} = (1-\varphi_2)\left[1-\varphi_1+\varphi_1\frac{\rho_{s1}}{\rho_f}\right] + \varphi_2\frac{\rho_{s2}}{\rho_f}, \quad (10)$$

$$\frac{\mu_{hnf}}{\mu_f} = \frac{1}{(1-\varphi_1)^{2.5}(1-\varphi_2)^{2.5}}, \quad (11)$$

$$\frac{(\rho C_p)_{nf}}{(\rho C_p)_f} = (1-\varphi_2)\left[1-\varphi_1+\varphi_1\frac{(\rho C_p)_{s1}}{(\rho C_p)_f}\right] + \varphi_2\frac{(\rho C_p)_{s2}}{(\rho C_p)_f}, \quad (12)$$

$$\frac{k_{hnf}}{k_f} = \frac{k_{s2}+2k_{bf}+2\varphi_2(k_{s2}-k_f)}{k_{s2}+2k_{bf}-2\varphi_2(k_{s2}-k_f)}, \quad \text{where} \quad \frac{k_{bf}}{k_f} = \frac{k_{s1}+2k_f+2\varphi_1(k_{s1}-k_f)}{k_{s1}+2k_f-2\varphi_1(k_{s1}-k_f)}, \quad (13)$$

$$\alpha_{hnf} = \frac{k_{hnf}}{(\rho C_p)_{hnf}}. \quad (14)$$

Here $\rho_f$ is the base fluid density, $\rho_{hnf}$ is the hybrid nanoparticle density, $k_{hnf}$ is the hybrid nanoparticle thermal conductivity, and $\alpha_{\text{hnf}}$ is the hybrid nanofluid thermal diffusivity. These values represent the Carreau hybrid nanofluid volume proportion of nanoparticle$\varphi_1$ and $\varphi_2$ ($if$ $\varphi_1 = 0$ and $\varphi_2 = 0$ correspond to a base fluid). Within the context of the continuity equation (3), the stream function $\psi(r,x)$is introduced in order to fulfill the requirements.

$$u = \frac{1}{r}\frac{\partial\psi}{\partial r}, \quad v = -\frac{1}{r}\frac{\partial\psi}{\partial x}, \quad (15)$$

Where $\psi = \sqrt{u(x)\nu_f x}\, Rf(\eta)$, is the function of the dimensionless stream and

$$\eta = \frac{r^2-R^2}{2R}\sqrt{\frac{u(x)}{\nu_f x}}, \quad \theta(\eta) = \frac{T-T_\infty}{T_p-T_\infty}, \tag{16}$$

As an illustration, the following is provided: the chemical categories (A and B) of concentrations:

$$m(\eta) = \frac{a}{a_0} \text{and } n(\eta) = \frac{b}{b_0}, \tag{17}$$

Upon incorporating the designated transformation, the governing equations have the following form.

$$2D_1k\left(1+we^2\left(f''\right)^2\right)^{\frac{n-1}{2}} + D_1(1+2k\eta)f'''\left(1+we^2\left(f''\right)^2\right)^{\frac{n-1}{2}} + D_1(n-1)we^2\left(f''\right)^3k\left(1+\ we^2\left(f''\right)^2\right)^{\frac{n-3}{2}} + D_1(n-1)we^2\left(f''\right)^2f'''\left(1+\ we^2\left(f''\right)^2\right)^{\frac{n-3}{2}}(1+2k\eta) - \left(f'\right)^2 + \ ff'' = 0, \tag{18}$$

$$D_4(1+2k\eta)\theta'' + 2kD_3\theta' + D_3Prf\theta' + (1+2k\eta)\left[N_bn'(\eta).\theta'(\eta)\right] + (1+\ 2k\eta)\left[N_t\left(\theta'(\eta)\right)^2\right] = 0, \tag{19}$$

$$\frac{1}{Sc}\left((1+2k\eta)m'' + 2km'\right) + m'f - Lmn^2 = 0, \tag{20}$$

$$\frac{\delta}{Sc}\left((1+2k\eta)n'' + 2kn'\right) + n'f + Lmn^2 = 0, \tag{21}$$

The associated boundary conditions are

$$f(0) = 0,\ \theta(0) = 1,\ m'(0) = L_sm(0),\quad N_bm'(0) + N_t\theta'(0) = 0, \delta n'(0) = \frac{-L_sn(0)}{\nu_f},\ f'(0) = 1,\ f'(\infty) = 0, \theta(\infty) = 0, m(\infty) = 1, n(\infty) = 1. \tag{22}$$

The curvature parameter $k$, the Weissenberg number($We$), Prandtl number ($Pr$), Schmidt number ($Sc$), ratio diffusion coefficients ($\delta$), the Brownian motion ($N_b$), the thermophoresis ($N_t$) and the homogeneous-heterogeneous reactions ($Ls$) are all non-dimensional constants in equations (18) through (22), they are generally defined as follows.

**Nomenclature**

| | | |
|---|---|---|
| Curvature Parameter | $k$ | $\frac{1}{R}\sqrt{\frac{\nu_f l}{u_0}}$ |
| Weissenberg number | $We$ | $\frac{\tau^2r^2u_0^3x^2}{l^3\nu_f}$ |
| Prandtl number | $Pr$ | $\frac{\nu_f}{\alpha}$ |
| Schmidt number | $Sc$ | $\frac{\nu_f}{D_A}$ |
| Ratio of the diffusion coefficients | $\delta$ | $\frac{D_B}{D_A}$ |
| Brownian motion | $N_b$ | $\frac{\tau D_B(b_w - b_\infty)}{\nu}$ |
| Thermophoresis limiting factor | $N_t$ | $\frac{\tau D_T(T_w - T_\infty)}{\nu T_\infty}$ |
| homogeneous-heterogeneous reactions limiting factor | $Ls$ | $\frac{l_s}{D_A}\sqrt{\frac{\nu_f l}{u_0}}$ |
| - | $D_1$ | $\frac{1}{[(1-\varphi_1)^{2.5}(1-\varphi_2)^{2.5}](1-\varphi_2)\left[1-\varphi_1+\varphi_1\frac{\rho_{s1}}{\rho_f}\right]+\varphi_2\frac{\rho_{s2}}{\rho_f}}$ |
| - | $D_2$ | $\frac{1}{(1-\varphi_2)\left[1-\varphi_1+\varphi_1\frac{\rho_{s1}}{\rho_f}\right]+\varphi_2\frac{\rho_{s2}}{\rho_f}}$ |

| | | |
|---|---|---|
| - | $D_3$ | $(1-\varphi_2)\left[1-\varphi_1+\varphi_1\frac{(\rho C_p)_{s1}}{(\rho C_p)_f}\right]+\varphi_2\frac{(\rho C_p)_{s2}}{(\rho C_p)_f}$ |
| - | $D_4$ | $\frac{k_{s2}+2k_{bf}+2\varphi_2(k_{s2}-k_f)}{k_{s2}+2k_{bf}-2\varphi_2(k_{s2}-k_f)}$ |

(23)

We predict that in most of instances, the chemical kinds A and B are diffusion coefficients, it would be about same in size, so assume that diffusion coefficients$D_A$ and $D_B$ are equal($ie.,\delta = 1$), hence

$$m(\eta)+n(\eta)=1, \tag{24}$$

Equations (20) and (21) thus become the following

$$\frac{1}{Sc}\left((1+2k\eta)m^{''}+2km^{'}\right)+m^{'}f+\frac{N_t}{N_b}\left[(1+2k\eta)\theta^{''}(\eta)+2k\,\theta^{'}(\eta)\right]-Lm(1-m)^2=0, \tag{25}$$

Based on the specified boundary conditions

$$m^{'}(0)=Ls\,m(0),\ \ m(\infty)\longrightarrow 1. \tag{26}$$

**Skin friction coefficient**

The coefficient of skin friction $C_f$ is the relevant physical property. This describes surface drag. The shearing stress at the wall surface$\tau_{rx}$ defined as

$$\tau_{rx}=-\mu_{hnf}\left(\left(\frac{\partial u}{\partial r}\right)\left[1+\Gamma^2\left(\frac{\partial u}{\partial r}\right)^2\right]^{\frac{n-1}{2}}\right)_{r=R}=-D_2f^{''}(0)\left(1+we^2\left(f^{''}(0)\right)^2\right)^{\frac{n-1}{2}}, \tag{27}$$

Here $\mu_{nf}$ is the viscosity coefficient. The definition of the coefficient of skin friction is

$$C_f=\frac{2(\tau_{rx})_{r=R}}{\rho_f u(x)^2}, \tag{28}$$

Using equations (27) and (28) obtains the result.

$$C_f\sqrt{Re_x}=-2D_2f^{''}(0)\left(1+we^2\left(f^{''}(0)\right)^2\right)^{\frac{n-1}{2}}, \tag{29}$$

**Nusselt number**

The local Nusselt numeral$Nu$ is the physical quantity of interest. The heat flux at the wall's surface $q_w$ is given by

$$q_w=-k_{hnf}\left(\frac{\partial T}{\partial r}\right)_{r=R}, \tag{30}$$

The local Nusselt number is given by

$$Nu=\frac{(xq_w)_{r=R}}{k(T_p-T_\infty)}, \tag{31}$$

By the equation (30) in (31) obtained

$$\frac{Nu}{\sqrt{Re_x}}=-D_4\theta^{'}(0), \tag{32}$$

In equations (29) and (32) $Re_x$ represents the local Reynolds number defined by $Re_x=\frac{Xu(x)}{\nu_f}$.

**Formulation of Fuzzification**

This study also investigates the use of fuzzy notions to compare nanofluid and hnf. After the non-linear ODEs are transformed into FDEs, the volume proportion of the hnf is obtained as a TFN. The MATLAB initiative bvp5c is utilized to solve FDEs. The flow heat and rate are affected by an instantaneous change in the volume proportion of hnf. The heat transmission and flow rate of the hnfis determined only by these factors, as some academics assess that the volume consisting of nanoparticles are between [0.01-0.04], Since $\varphi_1$and $\varphi_2$depict the volume proportion of nanoparticles MnZn$Fe_2O_4$ and $Ni$Zn$Fe_2O_4$of a fuzzy context, it is performable to approach a challenging case by obtaining volume fractions as a TFN.The nanoparticle's characteristics are defined in Table 2. The volume

proportions of hnfutilized for this investigation are categorized as TFNs; these TFNs are then converted into $\alpha$-cut techniques, and $\alpha$-cut regulates the fuzziness of TFNs.

The interpretation of the fuzzy number at each $\alpha$-cut was found using the TFNs ranging from 0 to 1.The TFNs define the triangle membership functions of the fuzzy numbers. Using a fuzzy process and the $\alpha$-cut approach, the nonlinear ODEs (18), (19), and (25) were transformed into FDEs with their corresponding boundary constraints for fuzzy solutions.

$$2D_1k\left(1+we^2\left(\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^2\right)^{\frac{n-1}{2}}+D_1(1+2k\eta)\frac{d^3}{d\eta^3}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\left(1+we^2\left(\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^2\right)^{\frac{n-1}{2}}+$$
$$D_1(n-1)we^2\left(\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^3 k\left(1+we^2\left(\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^2\right)^{\frac{n-3}{2}}+$$
$$D_1(n-1)we^2\left(\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^2\left(1+we^2\left(\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^2\right)^{\frac{n-3}{2}}(1+2k\eta)-D_2B\left(\frac{d}{d\eta}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)-$$
$$\left(\frac{d}{d\eta}[f_1(\eta,\alpha),f_2(\eta,\alpha)]\right)^2+f\frac{d^2}{d\eta^2}[f_1(\eta,\alpha),f_2(\eta,\alpha)]=0, \quad (33)$$

$$D_4(1+2k\eta)\frac{d^2}{d\eta^2}[\theta_1(\eta,\alpha),\theta_2(\eta,\alpha)]+2kD_3\frac{d}{d\eta}[\theta_1(\eta,\alpha),\theta_2(\eta,\alpha)]+D_3Prf\frac{d}{d\eta}[\theta_1(\eta,\alpha),\theta_2(\eta,\alpha)]+(1+$$
$$2k\eta)\left[N_bn'(\eta)\frac{d}{d\eta}[\theta_1(\eta,\alpha),\theta_2(\eta,\alpha)]\right]+(1+2k\eta)\left[N_t\left(\frac{d}{d\eta}[\theta_1(\eta,\alpha),\theta_2(\eta,\alpha)]\right)^2\right]=0, \quad (34)$$

$$\frac{1}{Sc}\left((1+2k\eta)\frac{d^2}{d\eta^2}[m_1(\eta,\alpha),m_2(\eta,\alpha)]+2k\frac{d}{d\eta}[m_1(\eta,\alpha),m_2(\eta,\alpha)]\right)+f\frac{d}{d\eta}[m_1(\eta,\alpha),m_2(\eta,+\frac{N_t}{N_b}\left[(1+\right.$$
$$2k\eta d2\,d\eta 2\,\theta 1\eta,\alpha,\theta 2\eta,\alpha+2k\,dd\eta\theta 1\eta,\alpha,\theta 2\eta,\alpha-Lm1-m2=0, \quad (35)$$

The persistent boundary constraints.

$$f(\eta,\alpha)=0,\ \frac{d}{d\eta}[f_1(\eta,\alpha),f_2(\eta,\alpha)]=1,\theta(\eta,\alpha)=1,\ \frac{d}{d\eta}[m_1(\eta,\alpha),m_2(\eta,\alpha)=L_sm(\eta,\alpha),\ N_b\frac{d}{d\eta}[m_1(\eta,\alpha),m_2(\eta,\alpha)]+$$
$$N_t\frac{d}{d\eta}[\theta_1(\eta,\alpha),\theta_2(\eta,\alpha)],\ \frac{d}{d\eta}[f_1(\eta,\alpha),f_2(\eta,\alpha)]=0,\eta\to\infty,\theta(\eta,\alpha)=0,\eta\to 0,m(\eta,\alpha)=1,\eta\to 1 \quad (36)$$

**Method of solution**

The governing equations (18), (19), and (25) are extremely nonlinear when the boundary conditions (22) and (26) are taken into effect. To solve the non-dimensional regulating equations (18), (19), and (25), In conjunction with the bvp5c method, the strategy for the boundary value problem is utilized. in MATLAB. First, transform equations (18, (19), and (25) to first-order ODEs.

## RESULTS AND DISCUSSION

The principal intention of this segment is to illustrate the physical flow problem with graphical representations. We = 3, k = 0.5, m = 0.2, L = 0.5, Sc = 1.2, $N_t = 0.5$, and $N_b = 1.5$ are some of the flow parameters that are evaluated to examine their effects on the surface's skin friction coefficient, temperature,and flow velocity. Fig.3-5 presents the graphical results generated by the MATLAB software. This section addresses the influence of different materials on the non-dimensional moment, energy, and concentration responses for both the nano fluid and hybrid nanofluid responses. A directive to assess the current results states that the Weissenberg numeral ($We$), Prandtl numeral ($Pr$), Schmidt numeral ($Sc$), curvature parameter ($k$), homogeneous-heterogeneous reactions parameter ($Ls$), Brownian motion($N_b$), and thermophoresis r ($N_t$) are different assumptions. Tabular data presents the outcome. Figure 6-7 depicts the fuzzy velocity and fuzzy temperature fields defined in the nanoparticles $\varphi_1$ and $\varphi_2$as TFNs.

Fig.3 (a) and (b)display a comparison between hybrid nanofluid and nanofluid velocity fields. The dotted lines show how hybrid nanoparticles change the behavior of the stretching cylinder, the velocity profile decreases for shear thinning ($n < 1$) fluid and increases for shear thickening ($n > 1$) fluid when the Weissenberg number is

enhanced. This is a significant observation. The fluid becomes denser and loses speed when the Weissenberg number increases, which represents the ratio of the fluid's relaxation time to the length of the specific procedure.Fig.3 (c) and (d) show how the curvature parameter (k) reduces the velocity profiles for shear thinning ($n < 1$) and shear thickening ($n > 1$)fluids in both circumstances. This is because the radius of the cylinder and the curvature limiting factor are inversely correlated. Thus, the cylinder's parameter decreases the radius by increasing curvature, which likewise decreases the surface. Consequently, the barrier to fluid movement is reduced, leading to an increase in velocity. The creation of lowered velocity curves was caused by the Carreau hybrid nanofluid particles' difficulties when this force had a resistive nature.On a physical level, thecurve sketched parameter is inversely related to the base fluid/hybrid nanoparticles'kinematic viscosity. These factors include the Weissenberg number (We), curvature (k),and the impact of constant parameters such as $k = 0.5, Pr = 2, m = 0.2, L = 0.5$, and $Sc = 1.2$.

Fig. 4(a) and (b) show a comparison of hybrid nanofluids with nanofluidsfor $We = 3, Pr = 2, m = 0.2, L = 0.5, Sc = 1.2, N_t = 0.5$, and$N_b = 1.5$. Increasing the Prandtl numeral ($Pr$) the temperature profile of hybrid nanofluidsreducesfor both shear thickening ($n > 1$) fluid and shear thinning ($n < 1$) fluids. This is caused by the presence of an inverse relationship between the Prandtl number and the diffusivity of heat. Hence, a larger value of the Prandtl numeral is related to low diffusion of energy as a result temperature declines and showssignificance identical in both hybrid nanofluid and nanofluid. Fig. 5 and 6 depict the volume proportion of nanoparticles of $MnZnFe_2O_4(\varphi_1)$ and $NiZnFe_2O_4$ ($\varphi_2$) that are classified as TFNs (see Table 2), and for specific values of $\alpha-$ cut, $\alpha = 0,0.3,0.7$and 1, $f'(\eta,\alpha)$ and $\theta(\eta,\alpha)$ are regulated by$\alpha - cut$.The fuzzy velocity $f'(\eta,\alpha)$ in Fig. 5(a) converts to the lower and upper boundaries when $\varphi_1$ is a TFN and the dashed line represents the hybrid nanofluidat $\varphi_2 = 0.03$, and the solid represents the nanofluid. In Fig. 5(b)$f'(\eta,\alpha)$converts into the lower and upper boundaries of the fuzzy velocity profiles when $\varphi_2$ is a TFN and the hybrid nanofluid at $\varphi_1 = 0.03$ is represented by the dashed line, while the solid represents the nanofluid. The width between the hnf and nanofluid diminishes in both scenarios when $\alpha$-cut increases, and they approach consistent at $\alpha - cut = 1$.In Fig.6 (a) the upperand lower boundaries of the fuzzy temperature profile are represented by the $\theta(\eta,\alpha)$ when $\varphi_1$ is a TFN and $\varphi_2 = 0.03$, the hybrid nanofluid is represented by the dotted line, a solid line indicating the base fluid. In Fig.6 (b)$\theta(\eta,\alpha)$transforms into the least and greatest bounds of the fuzzy temperature profile when a TFN is$\varphi_2$ and given $\varphi_1 = 0.03$, the hnf is represented by a dashed line and the base fluid by the solid line. The hybrid nanofluid and nanofluid become less wide at $\alpha - cut = 1$ and they become coherent with each other in both scenarios as $\alpha - cut$increases.

Fig 7shows the surface drag force against the curvature parameter. The size of the coefficient of skin friction has been shown to reduce along with increasing Weissenberg numeral($We$) values. In addition, the hybrid nanofluid exhibits skin friction values that are higher than those of the nanofluid. Fig 8The Nusselt numeral varies concerning the curvature limiting factor ($k$) and the Prandtl number ($Pr$). It has been shown that the Nusselt numeral is smaller when $Pr$ values increase. Moreover, compared to nanofluid, the hnf has a higher Nusselt numeral.

Using a membership function compares the hybrid nanofluid($MnZnFe_2O_4/NiZnFe_2O_4$) to the base fluid spanning wide ranges of value consider at$\eta = 0.3$. Here, TFN is calculated using the volume proportion of nanoparticles. Fig.9The hybrid nano fluid's velocity profile is found to be significantly less than that of the base fluid for shear thinning ($n < 1$) and greater for shear thickening($n > 1$), despite some fluctuation in the velocities of the two fluids. Fig.10there is no longer a noticeable temperature difference between base and hybrid nanofluids. For shear thinning ($n < 1$), hybrid nanoparticles can significantly diminish the base fluid's thermal conductivity; for shear thickening ($n > 1$), hybrid nanoparticles can significantly enhance the base fluid's thermal conductivity.

Table 3: In addition to the limiting case of parameters$m = 0.2$, $Pr = 2, L = 0.5$,and$Sc = 1.2$, When comparing the variation of skin friction values for different nanofluids and hybrid nanofluids with varying Weissenberg (We) numbers and curvature (k) parameters, it is evident that the coefficient of skin friction is often higher in hybrid nanofluids compared to regular nanofluids. If the curvature parameter value were to increase, the skin friction values of the hnf would be lower than those of the nanofluid.

Table 4: Describe the limiting cases with the parametersm $= 0.2$, $\mathrm{We} = 3, \mathrm{L} = 0.5$, 5and$\mathrm{Sc} = 1.2$, as well as the effect of the local Nusselt numeral on the difference between the nanofluid and hybrid nanofluid with various kinds of Prandtl ($Pr$) number and curvature (k) parameters. In some cases, the hybrid nanofluid's local Nusselt number value is improved than that of the base fluid.

## CONCLUSION

The significance of the present flow problem being studied is the periodic body velocity, temperature, and concentration for the flow of a modified Buongiorno model-based Carreau hybrid nanofluid along a stretched cylinder in a fuzzy environment. The governing equations were converted into a set of FDEs, which were then solved using MATLAB. We learned, that by fixing the parameters, the convergence of the approach was verified quantitatively. Uncertainty variability is examined using the triangle membership function (TMF), and the TFNs are governed by $\alpha$ - cut.

1. The hybrid nanoparticles' appearance in the fluid velocity compared with the nanofluid momentum causes the fluid velocity to decreasefor shear thinning $(n < 1)$fluid and increasefor shear thickening $(n > 1)$ fluid, in opposition to enhanced Weissenberg number $(We)$.
2. In the enhanced curvature factor, the velocity of the hybrid nanoparticles and nanofluid is fewer forboth shear thinning $(n < 1)$and shear thickening$(n > 1)$ fluid.
3. When the Prandtl number increases, the thermal conductivity of the nanofluid and hybrid nanofluid decreases in the same way for both shear thinning $(n < 1)$ fluid and shear thickening $(n > 1)$ fluid.
4. The consistent magnitude of the fuzzy velocity and temperature signifies the anticipated sensitivity of the TFN. As $\alpha$ increase from 0 to 1, the optimal response is situated between the upper and lower values.
5. Moreover, it is also clear that hazy triangle values that correspond to temperatures and velocities at various locations along the divergence channel were obtained. Also, temperature and velocity bounds were displayed at different times in the divergence channel's fixed value of the associated parameter.
6. The skin friction coefficient value is higher in the hnf than the nanofluid through parameters $k$ exhibited when the Weissenberg number$(We)$ increased.
7. The Nusselt number (Nu) of the hnf exceeds that of the nanofluid through the parameters k when the Prandtl number $(Pr)$ increases.

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**Table 1: Thermal physical characteristics of base fluids and nanoparticle**

| Thermal properties | $C_p(Jkg^{-1}K^{-1})$ | $\rho(kgm^{-3})$ | $k(Wm^{-1}K^{-1})$ |
|---|---|---|---|
| $MnZnFe_2O_4$ | 1050 | 4700 | 3.9 |
| $NiZnFe_2O_4$ | 710 | 4800 | 6.3 |
| $C_nH_{2n+2}$ | 1.69 | 850 | 0.145 |

**Table 2: TFNs of fuzzy hybrid nanoparticles of volume fraction**

| **Fuzzy number** | **Crisp value** | **TFN** | **$\alpha-$ cut approach** |
|---|---|---|---|
| $\varphi_1$ | $[0.01-0.04]$ | $[0, 0.05, 0.1]$ | $[0.05\alpha, 0{,}1-0.05\alpha], \alpha \in [0\ ,1]$ |
| $\varphi_2$ | $[0.01-0.04]$ | $[0, 0.05, 0.1]$ | $[0.05\alpha, 0{,}1-0.05\alpha], \alpha \in [0\ ,1]$ |

**Table: 3Coefficient of Skin frictions**

| $k$ | $We$ | $-C_f\sqrt{Re_x}$ $\varphi_1 = 0.03, \varphi_2 = 0$ NanoFluid | $-C_f\sqrt{Re_x}$ $\varphi_1 = \varphi_2 = 0.03$ Hybrid Nanofluid |
|---|---|---|---|
| 0.2 | 1 | 1.0363 | 1.0412 |
| | 1.5 | 0.8540 | 0.8597 |
| | 2 | 0.7327 | 0.7383 |

| | | | |
|---|---|---|---|
| 0.3 | 1 | 1.0950 | 1.0954 |
| | 1.5 | 0.8899 | 0.8928 |
| 0.5 | 1 | 1.2094 | 1.2017 |
| | 2 | 0.8079 | 0.8076 |

**Table: 4The Nusselt number**

| $k$ | $Pr$ | $-Nu/\sqrt{Re_x}$ $\varphi_1 = 0.03, \varphi_2 = 0$ NanoFluid | $-Nu/\sqrt{Re_x}$ $\varphi_1 = \varphi_2 = 0.03$ Hybrid Nanofluid |
|---|---|---|---|
| 0.2 | 1 | 1.2342 | 1.2931 |
| | 1.3 | 1.1651 | 1.2196 |
| | 2 | 0.9993 | 1.0430 |
| 0.3 | 1 | 1.3968 | 1.4571 |
| | 3 | 0.1928 | 0.1936 |
| 0.5 | 1.3 | 1.6524 | 1.8556 |

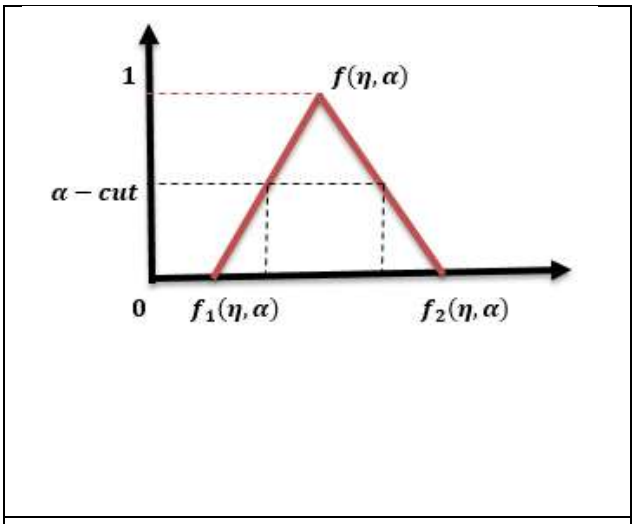



**Fig.1: MFs of a triangular fuzzy number.**

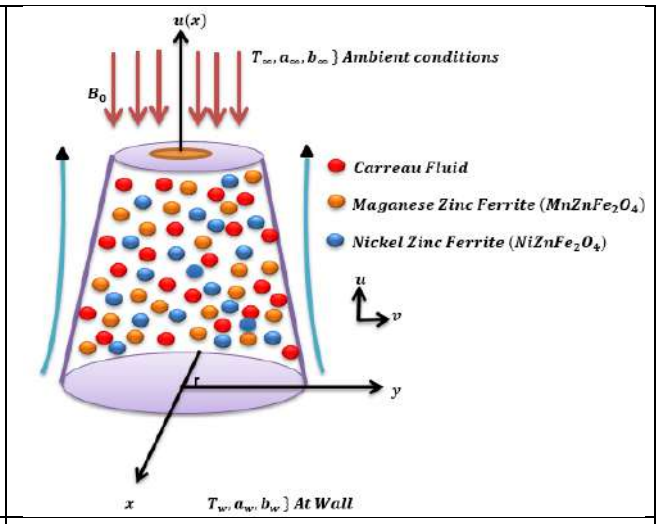
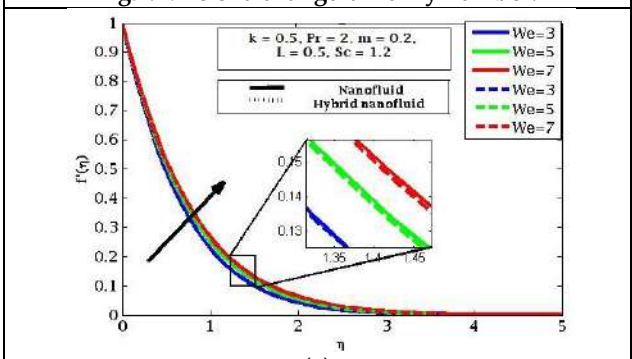
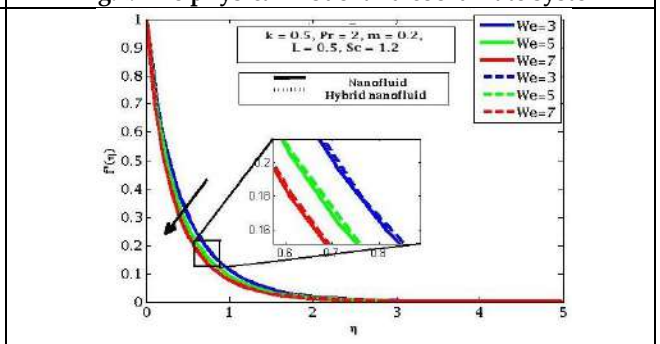



**Fig.2: The physical model and coordinate system**

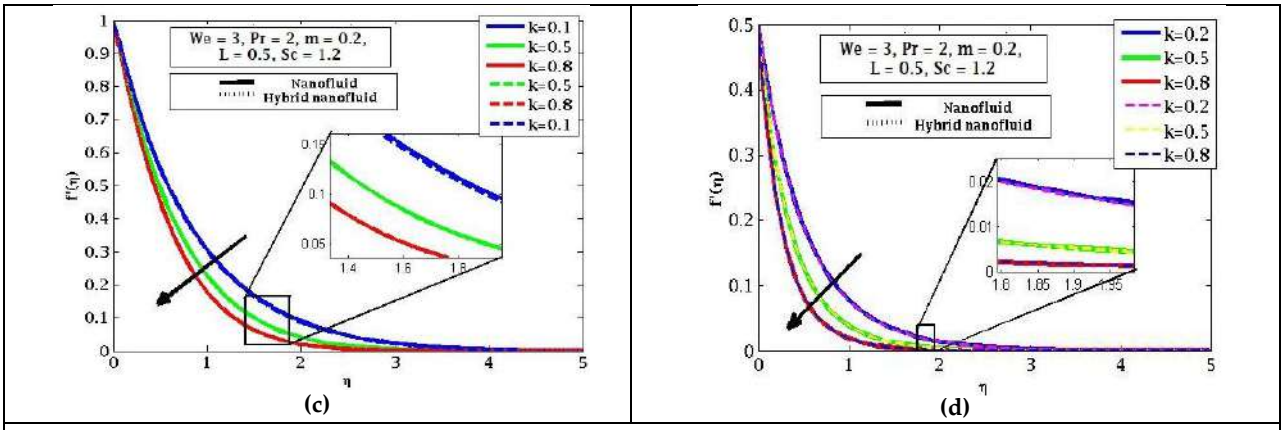



**(a)**



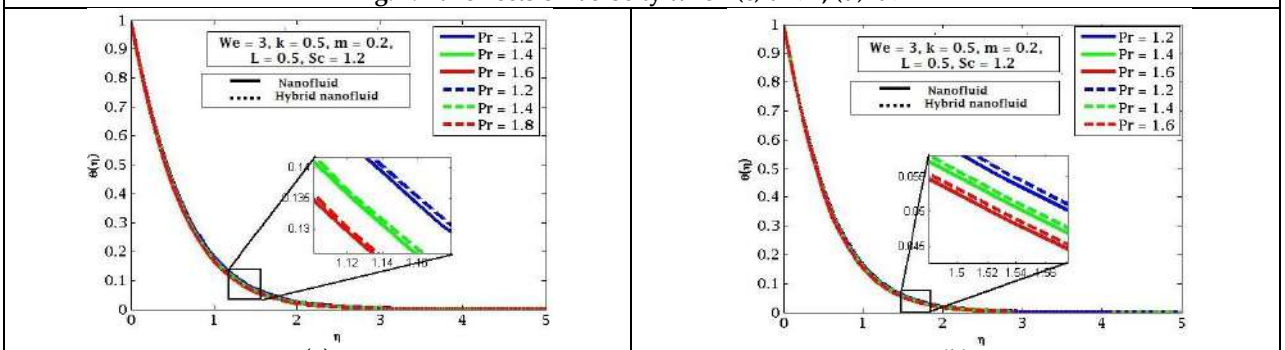

**(b)**

**Fig. 3: "$We$" effects on velocity when (a)$n < 1$, (b) $n > 1$**



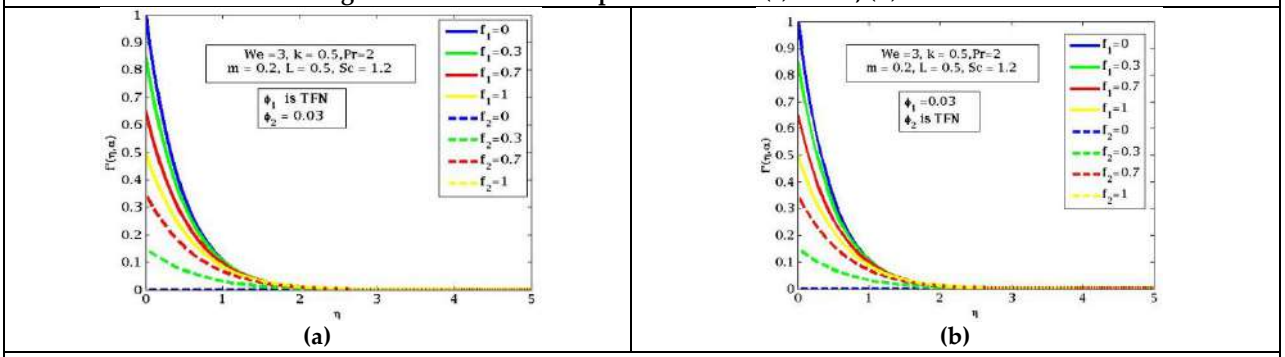

(c) (d)

**Fig.4 : "$k$" effects on velocity when (c)$n < 1$, (d) $n > 1$**



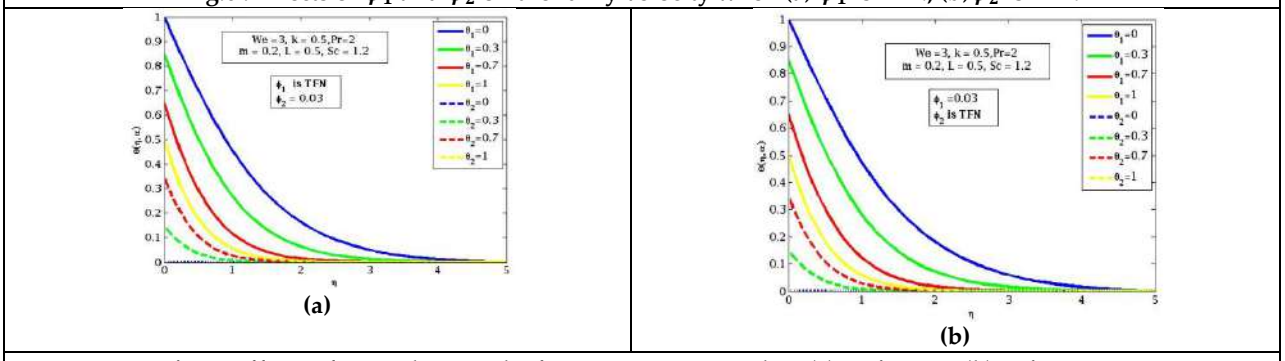

(a) (b)

**Fig. 5: "$Pr$" effects on temperature when (c) $n < 1$, (d) $n > 1$**




(a) (b)

**Fig.6 : Effects of $\varphi_1$ and $\varphi_2$ on the fuzzy velocity when (a) $\varphi_1$ is TFN, (b)$\varphi_2$ is TFN**




(a) (b)

**Fig. 7: Effects of $\varphi_1$ and $\varphi_2$ on the fuzzy temperature when (a) $\varphi_1$ is TFN, (b)$\varphi_2$ is TFN**

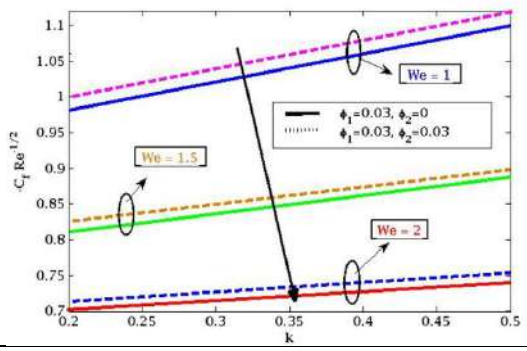



**Fig. 8. Effect of hybrid nanoparticles for on skin friction for different values**

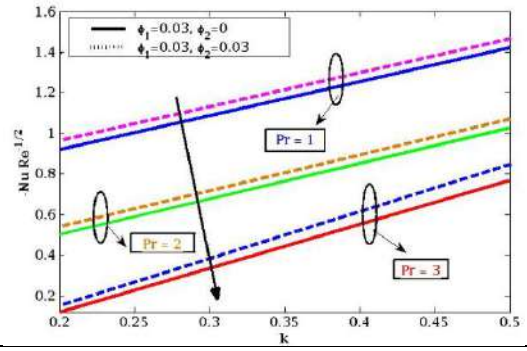
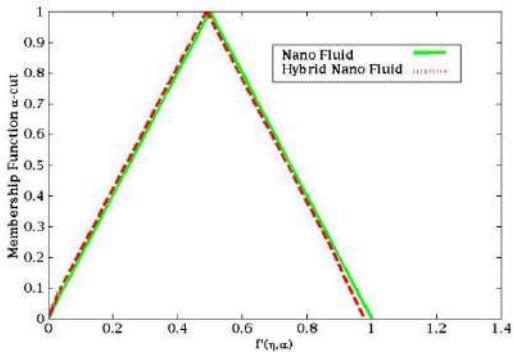
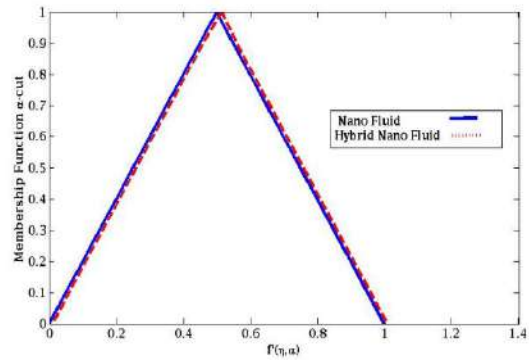



**Fig. 9. Effect of hybrid nanoparticles for on Nusselt number for different values**

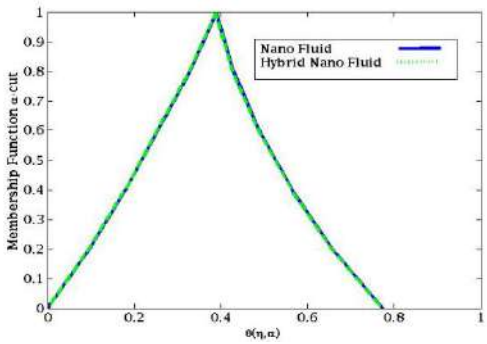



**(a)**

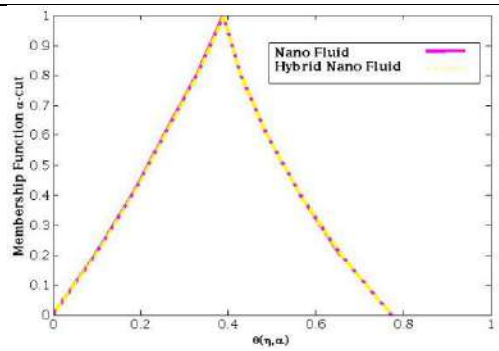



**(b)**

**Fig. 10. Comparison of nanofluid and hybrid nanofluid on fuzzy velocity at $\eta = 0.3$when (a) $n < 1$and (b) $n > 1$**




**(a)**




**(b)**

**Fig.11. Comparison of nanofluid and hybrid nanofluid on fuzzy temperature at $\eta = 0.3$when (a) $n < 1$ and (b) $n > 1$**

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**RESEARCH ARTICLE**

# Isolation, Identification and Bioprofiling of Endophytic Fungi from *Piper schmidtii*

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**ABSTRACT**

Endophytic fungi, *Neofusicoccum parvum* and *Pestalotiopsis microspora*, were isolated from the leaves of *Piper schmidtii* (a medicinal plant) and were confirmed by 18S rRNA gene sequencing. Compared with *Pestalotiopsis microspore*, the ethyl acetate extract of *Neofusicoccum parvum* revealed substantial levels of total phenolics (185.96 mg GAE/g extract), tannins (188.43 mg GAE/g extract), and flavonoids (25.81 mg QE/g extract), which are known for their antioxidant properties. These bioactive compounds exhibited significant activity in DPPH radical scavenging, ABTS, phosphomolybdenum reduction, and nitric oxide assays. Furthermore, the extract demonstrated notable $\alpha$-amylase inhibitory activity ($IC_{50}$: 65.51µg/mL), suggesting its effect in anti-diabetic.

**Key words:** *Piper schmidtii*, antioxidant property, antidiabetic potential, bioprofiling, $\alpha$-amylase inhibition

## INTRODUCTION

Traditional medicinal plants are essential sources for new pharmaceuticals due to their rich chemical diversity [1]. Concerns over side effects and bacterial resistance associated with synthetic drugs have highlighted the therapeutic potential of phytochemicals in herbal medicines [2]. Similarly, safety and efficacy issues with synthetic antioxidants have increased interest in natural alternatives, such as those from endophytic fungi [3].Endophytic fungi, living symbiotically within plants, are rich sources of bioactive compounds with antiviral, antifungal, anticancer, and antibacterial properties [4]. They often produce the same compounds as their host plants, possibly through genetic

exchange [5, 6,7]. The demand for antioxidants to combat free radicals is growing due to today's environmental challenges. *Piper schmidtii*(*Piperaceae*) holds significant importance in traditional medicine due to the presence of piperine, a compound with diverse ability such as antifungal, antioxidant, anti-inflammatory, antimicrobial, and antidiabetic [8,9].This study will investigate the bioactive potential of two endophytic fungi from *Piper schmidtii*. Despite their recognized benefits, global studies on the phytochemical composition and pharmacological activities of these fungi are scarce.

## MATERIALS AND METHODS

**Isolation of Endophytic Fungi**

Fresh plant materials were collected from TNAU, Coimbatore, Tamil Nadu, Indiaand identified by Botanical survey of India, Coimbatore (BSI/SRC/5/23/2022/Tech./404). Surface sterilized leaf segments were incubated on PDA with Chloramphenicol (150 mg/L) at 26±2°C for 15-20 days and the emerging fungi were identified based on conidial and spore morphology under a microscope(40X)[10].

**Molecular Identification**

The genomic DNA was isolated, and the ITS region was amplified using PCR with ITS-4 and ITS-5 primers [11]. These sequences were analyzed using BLAST in NCBI. Further, multiple sequence alignment and phylogenetic analysis were performed using Clustal X and MEGA X, respectively [12].

**Production of Bioactive Compounds**

Two fungal colonies (6 mm) were subcultured in PDB for 21 days. After incubation, the broth was filtered using Whatman No. 1 filter paper. The filtrate was mixed with an equal volume of ethyl acetate in a separating funnel and allowed to settle for 2h. The ethyl acetate extracts were then concentrated using a vacuum rotary evaporator at 40°C and stored at 4°C [13].

**Quantification of Bioactive Compounds**

The total phenolic content (TPC) was determined [14].The total tannin content (TTC) was measured [15]. The total flavonoid content (TFC) was assessed using colorimetric method [16].

***In vitro* Antioxidant Activity**

The free radical scavenging activity of fungal extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [17].The activity was expressed as the $IC_{50}$, which is the concentration needed to inhibit 50% of the DPPH radicals.The Modified ABTS assay, measures the scavenging ability of antioxidants on $ABTS^+$ free radicals. Antioxidant activity was expressed as Trolox equivalent (μM/g)[18].Phosphomolybdenum assay was evaluated [19].Nitric oxide assay was measured [20].

**Antimicrobial and Antifungal Activity**

The antibacterial study of endophytic fungal extract was evaluated against*Klebsiella pneumoniae* (*MTCC10309*), *Streptococcus pneumoniae* (*MTCC405*), *Pseudomonas aeruginosa* (*MTCC2057*), and *Staphylococcus aureus* (*MTCC9760*) using a modified agar disc diffusion method [21]. *Candida albicans* and *Aspergillusniger* were seeded on SDA plates. Discs with fungal extracts,negative control (DMSO) and a positive control (Fluconazole at 10 μg per disc) were placed on the agar plates.Zones of inhibition were observed 3-5 days after incubation at 28°C [22].

***In vitro* anti-diabetic activity**

The $\alpha$-amylase inhibition assay was determined[23].

## RESULTS AND DISCUSSION

**Morphological Characteristics**

*Neofusicoccum parvum* and *Pestalotiopsis microspora,* endophytic fungi isolated from *Piper schmidtii* leaves. *Neofusicoccum parvum* is characterized by its rapid growth with white aerial mycelia that darken with age (Fig. 1) The hyaline, aseptate conidia of *Neofusicoccum parvum* are oval to ellipsoidal, measuring 17.5–25.0 μm × 7.5–10.0 μm. Additionally, the branched, septate conidiophores of *Neofusicoccum parvum* produce pycnidia on PDA, measuring 370.6–908.9 μm × 234.5–506.4 μm. *Pestalotiopsis microspora* exhibited white to pinkish or brownish color colonies, with hyaline, aseptate conidia measuring 4-8 μm and pycnidia measuring 200-900 μm x 200-500 μm(Fig. 1).

**Molecular Identification**

Molecular identification using 18S rRNA gene sequences and ITS primers (ITS1 and ITS4) yielded 632 bp products (Fig.2). BLAST analysis was confirmed by the presence of *Neofusicoccum parvum* and *Pestalotiopsis microspora* with 99.82% and 99.44% identity to known sequences. Accession numbers were obtained upon sequence submission to NCBI GenBank (Table 1).

**Quantification of Bioactive Compounds**

*Neofusicoccum parvum* expressed a significantly higher total phenolic content (185.96 mg GAE/g) compared to *Pestalotiopsismicrospora* (60.81 mg GAE/g) (Fig.3).Comparison with previous findings from [24] reveals lower phenolic levels in *P. oxalicum* (120.99 μg GAE/mg) and *M. guilliermondii* (23.40 μg GAE/mg), emphasizing the significant outcomes of this research. Phenolic compounds, characterized by aromatic rings and hydroxyl groups, are pivotal bioactive substances known for their antioxidant properties [25]. In contrast with *Pestalotiopsis microspora*(61.66mg GAE/g), *Neofusicoccum parvum* exhibited an elevated level of tannin content (188.43mg GAE/g) due to increased polymerization of polyphenolics. *Neofusicoccum parvum* showed increased flavonoid content (25.81 mg RE/g) than *Pestalotiopsis microspora* (21.73 mg/g). This result surpass the flavonoid content reported in the ethyl acetate extract of *Aspergillus fumigatus* (20.49 mg/g)[26]. Flavonoids are known for their anti-inflammatory, antimutagenic, antiallergic, antiviral, and anticarcinogenic properties, making them valuable natural antioxidants [27].

***In vitro* Antioxidant Assays**

*Neofusicoccum parvum* exhibited a better $IC_{50}$ value of 56.19 μg/ml compared to *Pestalotiopsis microspora*, which had an $IC_{50}$ value of 93.21 μg/mlin DPPH assay and these results suggest that *Neofusicoccum parvum* possesses a strong hydrogen-donating ability, indicating high antioxidant potential. The ABTS assay revealed that *Neofusicoccum parvum* showed a radical scavenging activity of 97.22 μg/ml, and *Pestalotiopsis microspora* exhibited 53.68 μg/ml. Although both fungi demonstrated antioxidant activity, their effectiveness was lower compared to rutin (144.16 μg/ml) and BHT (145.34 μg/ml).Phosphomolybdenum reduction assay, measured in ascorbic acid equivalents (AAE), was higher for *Neofusicoccum parvum* (149.58 mg AAE/g) compared to *Pestalotiopsis microspora* (86.26 mg AAE/g). The observed reduction in free radicals can be attributed to the electron or hydrogen ion transfer capabilities of phenolics and flavonoids present in the fungal extracts. The result shows significantly higher antioxidant activity than the previous study [28]. *Neofusicoccum parvum* revealed a maximum NO scavenging activity of 35.74% in comparison with *Pestalotiopsis microspora* (12.28%) (Fig. 4). While lower than the reference compounds BHT (62.96%) and rutin (62.47%), these findings demonstrate the potential of fungal extracts to inhibit nitrite formation by competing with oxygen. NO and reactive nitrogen species can damage cells and cause cytotoxicity [29]. This assay suggests that fungal extracts from *Piper schmidtii* may help reduce inflammation and oxidative stress-related diseases by scavenging nitric oxide (NO).

***In vitro* anti-diabetic activity**

*Neofusicoccum parvum* and *Pestalotiopsis microspora* expressed $\alpha$-amylase inhibitory activityin ethyl acetate extracts, with $IC_{50}$ values of 65.51 μg/mL and 79.6 μg/mL, respectively (Fig.5).Endophytic fungi extracts were sources of novel antidiabetic compounds [30].

**Antimicrobial activity**

*Neofusicoccum parvum*indicates zone of inhibition against *Streptococcus pneumoniae* (8 mm), *Staphylococcus aureus* (7 mm), *Pseudomonas aeruginosa* (18 mm), and *Klebsiella pneumoniae* (10 mm) at 5 mg/mL.*Pestalotiopsismicrospora* showed inhibition against *Streptococcus pneumoniae* (9 mm), *Staphylococcus aureus* (8 mm), *Pseudomonas aeruginosa* (9 mm), and *Klebsiella pneumoniae* (8 mm) at 5 mg/mL(Fig.6.). Endophyticfungi produce secondary compounds against pathogens in host plants, emphasizing the promise for biotechnological drug research[31,32]. A visible zone of inhibition against *C. albicans* (14 mm) and *A. niger* (10 mm) were observed in *Neofusicoccum parvum* and *Pestalotiopsis microspora* respectively(Fig.6). These fungi produce strong antifungal compounds for treating fungal infections [33, 34]. Tannins from plants exhibit antibacterial and antifungal properties [35].

## SUMMARY

*Neofusicoccum parvum*evidenced particularly high levels of total phenolics, tannins, and flavonoids, along with potent antioxidant and antimicrobial properties. Further research is recommended to isolate and characterize specific bioactive compounds, to elucidate their mechanisms of action through *in vivo* studies, and explore their efficacy as medicinal agents. This work contributes to the development of sustainable and effective natural medicines, addressing contemporary health challenges.

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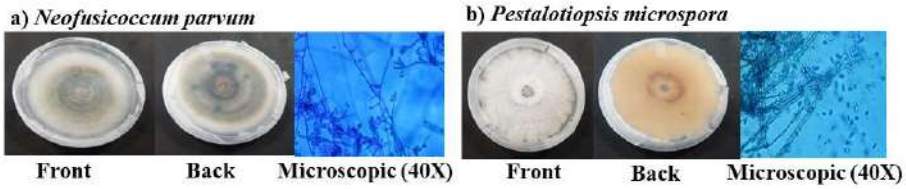
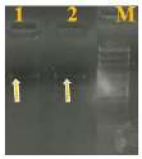
**Table 1: Blast Sequence**

| Plant | Endophytic fungi | Accession No | % Identity |
|---|---|---|---|
| *Piper schmidtii* | *Neofusicoc cumparvum* | PP703150 | 99.82% |
| | *Pestalotiopsis microspora* | PP703151 | 99.44% |

**Fig. 1: Morphology and Microscopic identification**






**Lane 1- *Neofusicoccum parvum***
**Lane 2- *Pestalotiopsis microspora***
**Marker- 100bp ladder**

**1. *Neofusicoccum parvum* (PP703150)**

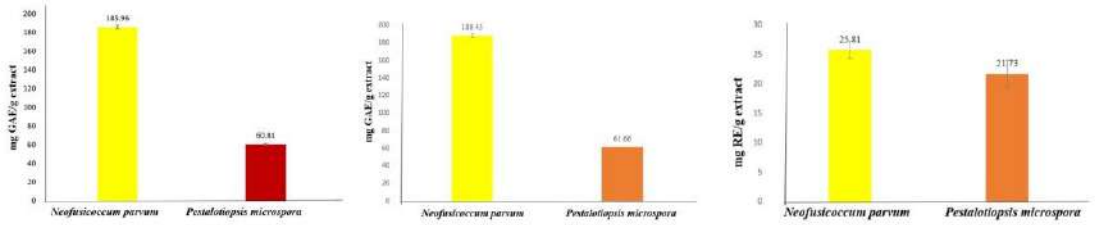
>ACCTGCGGAAGGATCATTACCGAGTTGATTCGAGCTCCGGCTCGACTCTCCCACCCAATGTGTACCTACCTCTGTTGCTTTGGCGGGCCGCGGTCCTCCGCACCGGC
GCCCTTCGGGGGGCTGGCCAACGCCCGCCAGAGGACCATAAAACTCCAGTCAGTGAACTTCGCAGTCTGAAAAACAAGTTAATAAACTAAAACTTTCAACAACGGATC
TCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTAT
TCCGAGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTATTGGGCCCCGTCCTCCACGGACGCGCCTTAAAGACCTCGGCGGTGGCGTCTTGC
CTCAAGCGTAGTAGAAAACACCTCGCTTTGGAGCGCACGGCGTCGCCCGCCGGACGAACCTTTGAATTATTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGCATATCAATAAGCCGGAG-567

**2. *Pestalotiopsis microspora* (PP703151)**

>TTCCGTAGGTGAACCTGCGGAGGGATCATTATAGAGTTTTCTAAACTCCCAACCCATGTGAACTTACCATTGTTGCCTCGGCAGAAGCTGCTCGGTGCACCTTACCT
TGGAACGGCCTACCCTGTAGCGCCTTACCCTGGAACGGCTTACCCTGCAACGGCTGCCGGTGGACTACCGAACTCTTGTTATTTTATTGTAATCTGAGCGTCTTATTT
TAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAAT
CTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAGCCTACTGCTTTTACTAGC
TGTAGCTCCTGAAATACAACGGCGGATCTGCGATATCCTCTGAGCGTAGTAATTTTTATCTCGCTTTTGACTGGAGTTGCAGCGTCTTTAGCCGCTAAATC-532

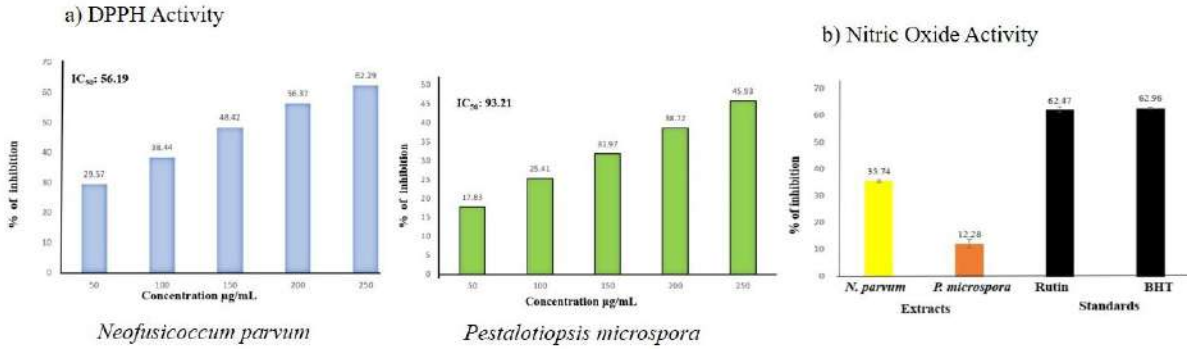
**Fig. 2: PCR Products**

**Fig. 3: Quantification of Bioactive Compounds**



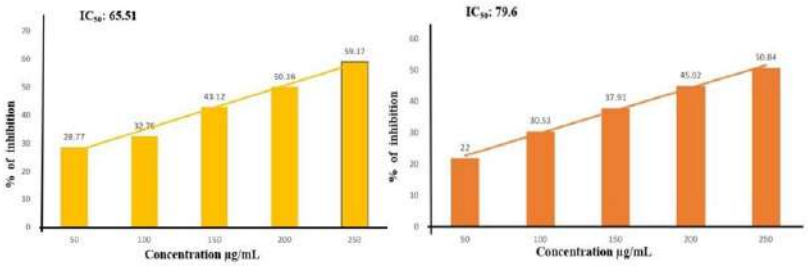

**a) Total phenolics** **b) Total tannins** **c) Total flavonoids**

**Fig. 4: *In vitro* Antioxidant Activity of Endophytic Fungi**




*Neofusicoccum parvum* *Pestalotiopsis microspora*

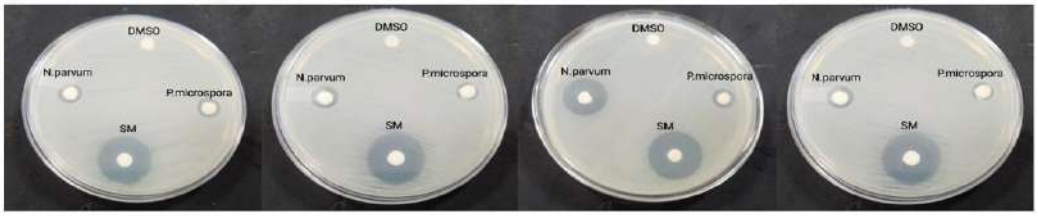
**Fig. 5: In vitro antidiabetic activity of Endophytic Fungi**




a) *Neofusicoccum parvum* b) *Pestalotiopsis microspora*

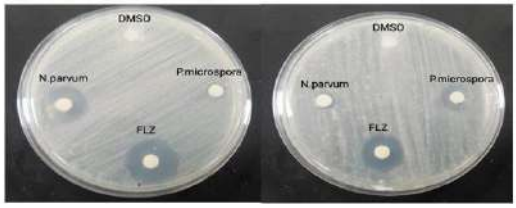
**Fig.6: Antimicrobial activity of Endophytic Fungi**

**a) Antibacterial activity**




*Streptococcus pneumoniae* *Staphylococcus aureus* *Pseudomonas aeruginosa* *Klebsiella pneumoniae*

**b) Antifungal activity**



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**RESEARCH ARTICLE**

# Mechanical Properties of Engineered Cementitious Composites Enhanced by Glass, Polyester Fibers and *Bacillus licheniformis* : An Experimental Approach

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**ABSTRACT**

Concrete is the primary material used in large-scale engineering projects because of its excellent compressive strength, even if it has a relatively low tensile strength. Engineered Cementitious Composites (ECC), often known as Bendable Concrete, is an innovative material that was created to enhance its Flexural and Tensile capabilities. Due to its high tensile ductility, toughness, and fine multiple cracking, ECC is a good choice for an environment friendly infrastructure that must meet strict requirements for longevity and safety. This research investigates the strength characteristics of various ECC mixtures by adjusting the quantities of supplementary cementitious materials, specifically polyester and glass fibers. The study utilized an $M_{45}$ ECC mixture with a water-to-binder ratio of 0.24 and a fly ash to cement ratio of 1.2 by mass. Bacillus Licheniformis microorganism are added in ECC with concentration of $10^7$ Cfu/g. Comparison is made between the compressive strengths of ECC mixtures with various fiber percentages and microorganism are compared to that of Conventional Concrete. Results show that bendable concrete exhibits higher Flexural and Tensile strengths than conventional concrete, underscoring the importance of glass fibers in enhancing ductility. polyester fibers in achieving the desired bendable concrete properties and microorganism in reducing the inherent voids of ECC.

**Keywords**: ECC, Glass Fiber, Polyester Fiber, Bacillus Licheniformis, Compressive Strength, Flexural Strength

## INTRODUCTION

Concrete remains the preferred material in large-scale engineering projects due to its remarkable compressive strength[1]. However, its relatively low tensile strength poses significant limitations, particularly in applications requiring high ductility and flexibility[2]. To address these deficiencies, Engineered Cementitious Composites (ECC), commonly referred to as Bendable Concrete, have been developed. ECC is a class of high-performance fiber-

reinforced cementitious composites characterized by high tensile ductility, toughness, and fine multiple cracking, making it an ideal candidate for sustainable infrastructure that demands longevity and safety [3] . The incorporation of fibers into ECC significantly enhances its mechanical properties. Traditional ECC formulations often utilize polyvinyl alcohol (PVA) fibers to achieve the desired flexibility and tensile strength[4]–[6]. However, recent studies have explored alternative fibers, such as polyester and glass fibers, which not only improve the mechanical properties but also contribute to the material's sustainability [7][8]. Polyester fibers are noted for their cost-effectiveness and environmental benefits, while glass fibers provide superior tensile strength and durability[9][10].

This study aims to investigate the strength characteristics of various ECC mixtures by altering the proportions of additional cementitious materials, specifically polyester and glass fibers. For the investigation, an M45 ECC mixture with a water-to-binder ratio of 0.24 and a fly ash to cement ratio (FA/C) of 1.2 by mass was chosen. By comparing the compressive strengths of ECC mixtures with varying fiber percentages to that of conventional concrete, this research seeks to establish the efficacy of polyester and glass fibers in enhancing the mechanical properties of ECC. The outcomes of this study are expected to contribute to the ongoing development of high-performance, sustainable construction materials that meet the rigorous demands of modern infrastructure[11]–[14]. This research aligns with current trends in material science, focusing on the utilization of innovative materials to improve the performance and sustainability of concrete structures [15].

## Material Parameters

Various materials such as Cement, Fly ash, Aggregates, Polyester Fiber, Glass fiber, Super plasticizer and water are used in ECC.

## Cement

In this study, OPC 53 grade cement was utilized as the binding agent for the concrete. The cement underwent evaluation based on various parameters as outlined in IS: 4031-1988[16] and was confirmed to comply with the standards set by IS: 12269-1987[17]. The physical properties of the cement are detailed in Table 1.

### Fly ash

Pulverized coal-based thermal power plants generate fly ash as a byproduct. Although fly ash is an environmental pollutant, it also holds potential as a valuable resource. It is frequently utilized in cement-based applications. The chemical properties of fly ash are presented in Table 2.

### Fine Aggregates

Natural Sand of 4.75 mm size is used as fine aggregate. To eliminate any particles larger than 4.75 mm and dust, the sand was first sieved using a 4.75 mm sieve. Sand properties were determined by conducting tests in accordance with IS 2386. (Part - 1)[18]. This project employed locally available 20mm size coarse aggregate. The fine and coarse aggregates were tested in accordance with IS: 383-1970[19]. Table 3 describes Sand- Physical Properties.

### Chemical Admixture

To improve the workability of the ECC mixture, PROCRETE R20, a polycarboxylic ether-based high-performance super plasticizer, is utilized. This additive ensures better flow ability and mixing ease while preserving the mechanical properties. By minimizing the water content, PROCRETE R20 maintains the necessary water-to-binder ratio and achieves optimal workability. Table 4 lists the characteristics of chemical admixtures.[20]

### Glass Fibers

In this study, glass fiber was incorporated into the engineered cementitious composite (ECC) concrete. Glass fibers were chosen for their high tensile strength and durability, contributing to enhanced ductility and overall mechanical performance of the ECC. The inclusion of glass fibers also helps to control cracking and improve the composite's toughness[21]. Table 5 lists the Glass fibres' physical characteristics.

**Polyester Fibers**

Polyester fibers were incorporated into the Engineered Cementitious Composite (ECC) mixtures to enhance their mechanical properties. These fibers were selected for their cost-effectiveness, environmental benefits, and ability to improve the tensile strength and durability of the ECC. The inclusion of polyester fibers aims to achieve the desired flexibility and ductility in the composite material[22]. The physical parameters of Polyester fibres are listed in Table 6.

**Water**

Water is a necessary ingredient in the manufacture of concrete. It aids in the creation of cement gel strength. It complies with the standards of IS 456-2000. The pH of the water is 7 to 8. The temperature is from 27 to 29 $^{o}$C.

**Bacillus licheniformis**

The Bacillus licheniformis strain used in this study was sourced from an Aumenzymes laboratory. The bacteria were cultured in nutrient broth and incubated at 30°C for 24 hours to reach the exponential growth phase. After incubation, the bacterial suspension was centrifuged at 3000 rpm for 10 minutes to obtain a concentrated bacterial pellet. The pellet was then resuspended in sterile distilled water to achieve a final concentration of 107colony-forming units (CFU) per gram.

**Experimental investigation**

Various experiments were conducted using 0.015 $m^3$ of concrete to determine the optimal percentage for the ECC mix design, based on insights from prior research. This study utilized an $M_{45}$ grade ECC mixture with a fly ash to cement ratio (FA/C) of 1.2 by mass and a water/binder ratio of 0.24. The primary objective was to evaluate the enhanced characteristics of ECC by varying the proportions of glass and polyester fibers. The compressive and flexural strengths of multiple ECC mixes with different fiber contents were compared to those of the Indian Standard Conventional Concrete design.

**Preparation of ECC**

The preparation of Engineered Cementitious Composites (ECC) involved mixing Ordinary Portland Cement, fine sand, and fly ash, followed by the gradual addition of glass and polyester fibers for uniform distribution. A Bacillus licheniformis suspension, cultured to $10^7$ cfu/g, was mixed with the required water and added to the dry materials, along with a superplasticizer to enhance workability. The homogeneous mix was then cast into molds and initially cured under plastic sheets for 24 hours at 27°C. After demolding, specimens were water-cured at 27°C for 28 days. Mechanical strength was carried out confirmed the effectiveness of the composite. Increased of compressive strength is found by impregnation of fibers and microorganism. In this study, Glass Fiber 2% shows good results. Wherein, the compressive strength is increased by 10.66% compared to normal concrete at 28$^{th}$ day. Increased of Flexural strength is found by impregnation of fibers and microorganism. In this study, Glass Fiber 2% and Polyester Fiber 2% show good results. The Flexural strength of Glass Fiber 2% is increased by 59.7% compared to normal concrete at 28$^{th}$ day. The Flexural strength of Polyester Fiber 2% is increased by 69.2% compared to normal concrete at 28$^{th}$ day.

## CONCLUSION

The following findings on the hardened characteristics of ECC with Glass and Polyester fibres for $M_{45}$ grade concrete are developed based on experimental research. The super plasticizer was used at a dose of 0.8 percent of the total powder content in the provided combination. ECC with Glass fibres has a compressive strength of 10.66% more than conventional concrete. Coarse aggregates are not used in this study, so, compressive strength of Polyester fibers decreases. The overall weight of ECC decreases as coarse aggregates are not used. Glass fibre has a flexural strength increase of 59.7%, whereas Polyester fiber has enhanced flexural strength of 69.2 %. The Hardened strength properties of ECC increases after adding bacteria and fibers because the bacteria induce calcium carbonate precipitation, which fills micro-cracks and strengthens the matrix, while the fibers bridge cracks and distribute stress,

enhancing the composite's toughness and load-bearing capacity. This combination results in a significant improvement in both flexural and compressive strength, demonstrating the effectiveness of integrating biological and material science principles in enhancing the performance of cementitious composites. Hence, use of fiber and microorganism in Engineered Cementitious Composites shall bring many opportunities for sustainable development and preserving nature.

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**Table 1: Cement- Physical Properties**

| Test | | Value |
|---|---|---|
| **Settling Time (initial)** | | 40 min |
| **Settling Time (final)** | | 190 min |
| **Compressive Strength** | 3 days | 36.80 N/mm² |
| | 7 days | 43.82 N/mm² |
| | 28 days | 56.78 N/mm² |
| **Soundness** | | 1 mm |
| **Standard consistency** | | 27.25 % |
| | | |

**Table 2: Chemical properties of Fly ash**

| No | Test Details | Requirement as per IS 3812 {Part-I} | Test Result |
|---|---|---|---|
| **1** | $SiO_2 + Al_2O_3 + Fe_2O_3$ | > 70.00% | 89.08 |
| **2** | $SiO_2$ | > 35.00% | 68.25 |
| **3** | MgO | < 5.00% | 1.67 |
| **4** | $SO_3$ | < 3.00% | 1.13 |
| **5** | Cl | < 0.05% | 0.019 |
| **6** | Total alkali as $Na_2O$ | Max 1.5 | 0.167 |
| **7** | Loss on ignition (LOI) | < 5.00% | 0.79 |

**Table 3: Sand- Physical Properties**

| Characteristics | Fine Aggregates |
|---|---|
| **Fineness modulus** | 2.9 |
| **Specific Gravity of Sand** | 2.670 |
| **Water Absorption (%)** | 0.9 |
| **Size** | 4.75 max |
| **Moisture content (%)** | 1.8 |
| **Zone of sand** | II |

**Table 4: Properties of Chemical Admixtures**

| Test | Value |
|---|---|
| **Appearance** | Brown |
| **Specific Gravity** | 1.3 @ 20$^{o}$C |
| **Air Entrainment** | < 2% |
| **Chloride Content** | Nil |

**Table 5: Physical Properties of Glass Fiber**

| Test particulars | Value |
|---|---|
| **Diameter** | 20 μm |
| **Tensile Strength** | 2200 N/mm$^2$ |
| **Length** | 12mm |
| **Melting Point** | 1400 $^{o}$C |

**Table 6: Polyester Fiber- Physical Properties**

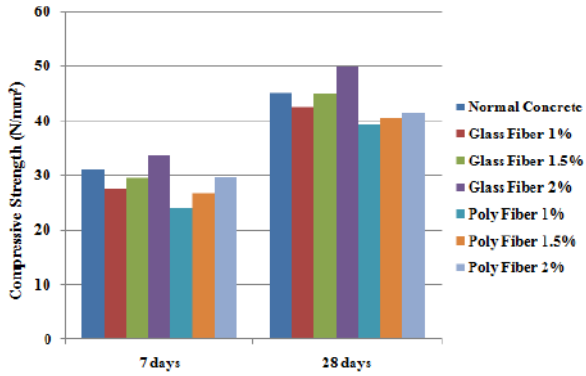
| **Test** | **Value** |
|---|---|
| **Length** | 6 mm |
| **Diameter** | 20 μm |
| **Elongation** | 33.3% |
| **Tensile strength** | 600 N/mm$^2$ |
| **Melting point** | 250-265 |
| **Density** | 1.38 $^{o}$C |
| **Aspect Ratio** | 6 |

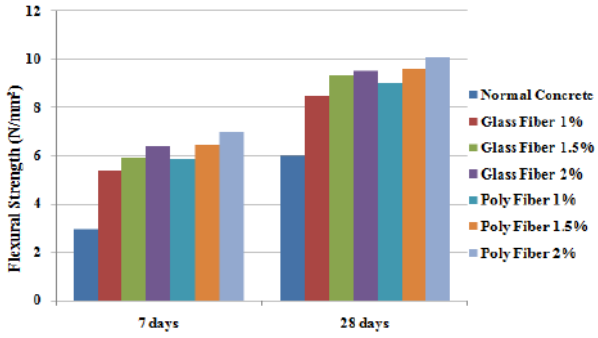
1.

2.

3. **Fig. 1: Glass Fibers**

4. **Fig. 2: Polyvinyl Alcohol Fibers**

5. 


6. 


7. **Fig. 3: Compressive Strength Results**

8. **Fig. 4: Flexural Strength Results**

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**RESEARCH ARTICLE**

# Social Life of Blacks in Claude McKay's *Home to Harlem*

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**ABSTRACT**

Festus Claudius McKay is a distinguished writer of African American literature. He was born in Jamaica in 1889 to peasant farmers whose strong sense of racial tradition was passed on to McKay through African folk tales and stories of his older brother, a school teacher and free thinker, from whom McKay learned the essence of maintaining his intellectual independence from authority. *Home to Harlem,* a famous picaresque novel in African American Literature. The first protagonist of the novel Jake is an army man. He wants to serve the country as a soldier. However, he fails to accomplish his goals due to color and racial prejudices. So, he escapes from the army and returns to Harlem for better life. Ray, the novel's second protagonist, he was alienated from the intellectual life of African American and American society. Thus, the novel depicts how the black men are suppressed and oppressed by the white society and how these young men as self-esteemed youngsters find immense pride and pleasure in retaining their inherited identity. Thus, the article focuses on how the blacks' life is socially meaned in the novel *Home to Harlem.*

**Keywords:** Picaresque, Self-esteemed, Suppressed, White society, and Black men

## INTRODUCTION

Literature is an artistic expression of humans to provoke his fellow beings in a certain way. It has the power of re-creating human psyche. It investigates profound knowledge to extend and access human credibility. The writers strongly believe that their writing is a weapon to change one's thoughts, feelings, and emotions. Many writers have proved their word power through their writings. Likewise, African American literature is one of the best examples for creating a big revolution to get back black people's equality and justice from color, racial prejudices, and colonial domination. African American writers strongly believed that their writings will definitely guide and offer solutions

to the blacks, to lead their life in a moral and successful way. They also believed that their words are like weapons, and through their words, they can give confidence to the blacks to encounter all kinds of racial, cultural, social, and political prejudices.

*Home to Harlem*, Claude McKay's debut novel, became the first most popular novel written by a Black author in 1928. It is a significant piece of practical social fiction and also one of McKay's outstanding novels. The novel is entirely a riot of exotic color and unrestrained indulgences. In the character of Ray, Jake's Haitian friend and the probable counterpart of the author, McKay portrays a sensitive, intelligent black who has distaste and sometimes an aversion for his low-life environment. His dream is to be a writer. Upset in his thinking by Russian Revolution and the World War I, he stands as a confused black man in a social order dominated by the whites.

The first protagonist of the novel Jake is an army man. He wants to serve the country as a soldier. However, he fails to accomplish his goals due to color and racial prejudices. So he escapes from the army and returns to Harlem for better life and love. Thus, the novel depicts how the black men are suppressed and oppressed by the white society and how these young men as self-esteemed youngsters find immense pride and pleasure in retaining their inherited identity.

**Proletarian Blacks in *Home to Harlem***

*Home to Harlem* contains an impressive critique of American and European imperialism by McKay. Jake Brown, the protagonist of the novel, is a tall, appealing man with dark brown complexion. Readers can understand how the black's transnational proletariat life attempts to help them regain their black identity through the character. The main purpose of the novel is to provide a broad overview of Harlem through Jake's international travels. Moreover, the narrative mostly recounts his experiences traveling abroad and living in Harlem. Naturally, someone with this kind of love for his own nation begins to recognize the richness and distinction of his race, color, and class. After garnering experiences from the transnational life, migrants feel proud of their country and find it better and more convenient than any other country.

Through an examination of the fundamental ways in which heterosexuality shapes the worldwide drama of war, race, and black people's longing towards home, McKay's vision of black social advancement and the domestic substance of the national romance are reconstructed in *Home to Harlem*. A queer reading of McKay's novels should be employed in addition to a gender analysis to investigate how sexuality structures and discourses impact the author's final departure from tales of home.

The novel's second central character, Ray, is a rational and scholar of Haitian descent. For the final two-thirds of the novel, he travels with Jake after they first meet on a train. The novel tells the story of their differences over and over again. Ray is an ingrained reader and aspiring writer who is a strong opponent of colonialism, despite feeling powerless to change the course of events. Unlike the gorgeous Jake, Ray is unsuccessful in his attempts to get close to women, and his drug experimentation puts him in the hospital. He laments the fact that his intelligence and bourgeois morality prevent him from being the "natural man" that Jake is.

The concept is, how the knowledge obstructs passion and resides at the heart of the primitivism that became popular in the 1920s. Liberated from the constraints of civilization, Black people could express their emotions directly, but White people's thoughts and morality suppressed them. McKay was one among the black writers embodied the positive remarks of primitivism as a centre of African thought. However, he ended up perpetuating stereotypes that were all too common, much like other people who attempted.

In McKay's *Home to Harlem*, primitivism is made political through his leftist radicalism and racial consciousness. It is a critique of white American morality and culture and, as such, a political statement of black identity. However, it also becomes a positive and particular technique of recovering and revaluing that is exclusive to African American

consciousness. Even with McKay's radical views, primitivism nevertheless reveals the work's immersion in the white authoritarian individualism ideology at the heart of the American myth.

The characters in Claude McKay's *Home to Harlem* play a minor role in American culture as well as the Harlem community. They lack education in the main. They reside in hotels that are now referred to as "single-room occupancy" and boarding homes. Their relationships are short-lived and tumultuous; they frequently terminate suddenly and occasionally violently. The women they deal with earn a living on their own income as entertainers, cooks, waitresses, domestics, or clerks; some of them also work as part-time prostitutes.

Helen Pyne-Timothy in "Perceptions of the Black Woman in the Work of Claude McKay," explains the issue of young black women engaging in prostitution, a problem that McKay handles with great sensitivity. Many of the young black women he notices have careers in this field. This type of black woman is ordinary in McKay's eyes, and he feels that he lends her significance in his art. He recognizes that the black community's economic situation has a big impact on how often these women are forced to live this kind of life. In order to survive, the black women are often found themselves on the streets whether they are in America or in Europe. Black women should not be deprived of their warmth and spontaneity by the prostitution industry, according to McKay, who particularly values these attributes in black women. The issues with black women's prostitution are evaluated in these two assessments. In *Home to Harlem*, there is fierce tenderness as well as rivalry between women and men.

**Social Life of Blacks in *Home to Harlem***

Black people's gathering spots are night clubs, cabarets, pool halls, saloons, buffet flats, houses of prostitution, and gambling dens. They smoke opium and sniff cocaine, and they consume alcohol in large quantities. In addition to the intense desire they have for freedom, they also live with the possibility of violence from white civilization. Drug addiction, sexual struggle, self-hatred, corruption, color-complexes, ghetto congestion, and class divisions among the African Americans are also depicted in *Home to Harlem*. Acknowledging these issues as the results of white atrocities against African Americans, McKay does not downplay them or offer an apology.

In *Home to Harlem*, McKay shows that even among the most disadvantaged African Americans, there remains a strong will to survive and reap the rewards of life, in spite of all the atrocities imposed upon them by white people. This fervent acceptance of life is occasionally justified by the worst parts of their experience. When Jake Brown, the protagonist of the novel, reminds other black workers which it is considered improper to cross picket lines and serve as "scabs," one black longshoreman angrily retorts those black workers are frequently denied union positions. According to Jake:

> Talking death, tha's what you sure is. One think I know is niggers am made foh life. And I want to live, boh, and feel plenty o' the juice o' life in mah blood. I wanta live and I wanta love. And niggers am got to work hard foh that. Buddy, I'll tell you this and I'll tell it to the wo'l'-all the crackers, and all them poah white trash, all the nigger-hitting and nigger-breaking white folks-I loves life and I got to live and I'll scab through hell to live. (Cooper xxiii)

Jake, Claude McKay's embodiment of the vitality and core values of African Americans, listens but withdraws. In Home to Harlem, Jake describes his picaresque voyage through "the semi-underworld" of black working-class America. Following his employment as a third cook in the Pennsylvania Railroad's dining car service, he contacts Ray, a waiter and a poor Haitian genius. As a friendship grows, McKay is able to contrast and balance each other's advantages and disadvantages against the backdrop of their "rough-and-tumble work and night life" (Cooper xxiv). Ray and Jake concur that despite severe restrictions, black people can express themselves positively through their shared love of dancing, music, and laughter, especially when they gather together for nighttime get-togethers. The black people appear to be having a great time with themselves, showcasing their own hard-earned colour, creativity, and happiness:

> Black lovers of life fixed in their own free native rhythm, threaded to a remote scarce-remembered past, for themselves, of themselves, in an exceedingly house in Fifteenth Street, Philadelphia. . .(Cooper xxiv).

*Home to Harlem* also admits the less hopeful fact that African American life in Harlem and other rising Northeast ghettos is unduly constrained and restricted, in contrast to these poetic elements of African American life as a dining-car waiter on the Pennsylvania Railroad during the First World War. These situations often result in anger and self-loathing, and self-harm puts the community as a whole at constant risk. While acknowledging the African American community's potential, McKay also highlights the internal pressures that pose a threat to the community's survival in *Home to Harlem*.

McKay starts a fictional quest in *Home to Harlem* for self-reliance, self-worth, and purpose in the stylish African American lifestyle that would consume him in later works. It is a challenged novel written by a writer whose own stresses and questions are never far from the surface, especially in light of all the bold declarations of African Americans' strength and happiness throughout. McKay characterizes Ray and Jake as hopeful, nevertheless. Their message of affirmation and hope, with a few standout exceptions, is a recurring theme in black literature, especially in light of the serious issues still plaguing America's black inner cities. Black folklore, according to McKay, ends in the country's cities where "men like Jake in *Home to Harlem* exemplify the great Migration northward that began in his day" (xxiv). If the African American community is to take charge of its own future and stop being a victim of American capitalism, which sees African Americans as lifeless pieces on a chessboard of gain and loss, then these men must be honoured for their hard reality and selfless attitude.

The first chapter of the novel titled as "Going Back Home" clearly expresses the protagonist's nostalgia for the home land. Jack experiences color and racial prejudices as well as alienation in his transnational life in Virginia and Europe.According to S.P. Adinarayan's definition in *The Case for Color*, color prejudice refers to a mindset or collective attitude toward specific individuals, primarily sparked by their race, particularly the color of their skin. In certain cases, it can also be reinforced and intensified by political, social, and economic factors, in addition to the innate emotions of jealousy, fear, and disgust. It is surcharged with emotion and is by its very nature irrational.

The novel's hero Jake, is a freighter stoker traveling from Cardiff, Wales to New York. His patriotic reasons lead him to enlist in the army in 1917, the year America declared war on Germany. He is upset, though, when he finds out against his wishes that he must transport building supplies for the barracks. He could witness racial discrimination even in the army. He is forced to perform laborious physical tasks instead of fighting, such as"lumber-boards, planks, posts, rafters-for the hundreds of huts built around Brest's walls and along cost between Brest and Saint-Pierre, to house the soldiers of the United States" (4). As a result, he makes the decision to leave the army, declaring that he will not be involved in the struggle against white political and economic empire.

*Free* by Mbanefo S. Ogene, Esther Chikaodi Anyanwu, and Ngini Josephine Ojiakuexpla in the article entitled "A Comparative Analysis of Racial Discrimination in Claude McKay's *Home to Harlem* and Kenneth Kaunda's *Zambia Shall be*," describe Jake's feelings of shame and resentment about the injustices done to Black people during World War II. The Yankees' treatment of Black people by calling them "niggers" or "darky" resents him. McKay observes:

> He knew that when a Yankee said Nigger, he meant hatred for the Negroes, whereas when he said 'darky,' he meant friendly contempt. He preferred white folks' hatred to their friendly contempt. To feel their hatred made him strong and aggressive, while their friendly contempt made him ridiculously angry, even against his own will. *(HH 5)*

McKay's contemporary writers Richard Wright and W.E.B. Du Bois in *The Souls of Black Folk* and *Native Son* respectively have also depicted such kind of discrimination in their works. Black people were routinely beaten and lynched by white people for no apparent reason. Even after the historic proclamation, white people still segregate and discriminate against Black people. W.E.B. DuBois states that:

> A black stranger in a Baker Country, Georgia, for instance, is liable to be stopped anywhere on the public highway and made to state his business to the satisfaction of any white interrogator. If he fails to give a suitable answer, or seem too independent or 'sassy,' he may be arrested or summarily driven away. (*The Souls of Black Folk*133)

This sentiment is also present in Richard Wright's Native Son, when the thieves exhibit dread due to their ingrained hatred of African Americans. Wright aptly states the pitiable plight of the blacks: "being dragged over the snow, his feet were up within the air, grasped by robust hands. 'Kill 'im!''Lynch 'im!''That black son of a bitch!''Kill black ape!'" (301). Black people in America are conditioned to believe that it is acceptable for them to take whatever the white people throw at them.

Jake leaves the army where he faced racial discrimination, dresses in civilian clothes, and travels to London to work on the docks. He spends time with a white girlfriend for the rest of the war. Jake is not happy with the white woman, so their relationship is unpleasant. Jake views the white woman as merely an alien being from a different planet. He begins to harbour feelings for Felice, a brown woman. Through his bizarre chat with the ship, Jake expresses his desire to get to Harlem as soon as possible. He conveys his wish to the ship even though he is fully aware that it is an absurd object that cannot react to his commands. This delves into his longing to return to his hometown of Harlem:

> Take me Home to Harlem, Mister ship!
> Take me Home to the brown gals waiting for
> the brown boys that done show their mettle
> Over there. Take me home, Mister ship. Put
> Your beak right into that water and jest move
> along…. (*HH* 9)

Jake's return trip to America is the first time that a physical representation of Harlem is mentioned. Jake's boredom and nostalgia disappear as soon as he gets to Harlem. Music,sex, and alcohol satisfy his insatiable thirst for joy. His desires get fulfilled in Harlem's Lenox Avenue:

> Harlem! Harlem! thought Jake. Where else could I have all this life but Harlem? Good old Harlem! Chocolate Harlem! Sweet Harlem! Harlem, I've got you' number down. Lenox Avenue, you're a beer, I know it. And, baby honey, sure enough youse a pippin for your pappy. Oh, boy! . . . (*HH* 14)

Jake finds it "crazy" to be walking around his neighbourhood once more: "He had been gone from Harlem for two years. He was being called by girls who were chocolate-brown and walnut-brown from One Hundred, Lenox Avenue, and Fifth Avenue and Thirty-Fifth Street"(8). Before he truly returns to his old neighbourhood, he has dreams about it. There are two aspects noted in this initial description. At first Jake's more desirable streets are 135 Street, Fifth Avenue, and Lenox Avenue. Second, in Jake's fantasy and imagination, the streets are filled with the brown women and their "tantalizing brown legs" (8). It is seen as sexual radicalization and he can rejoice himself in the city-streets.

Jake visits a saloon on his way back to his former neighbourhood. He is ecstatic to see Harlem as he subsequently "promenades" (10) along Seventh Avenue:"His blood was hot. His eyes were alert as he sniffed the street like a hound" (10). The metaphor of Jake as a hunting hound and his animalistic traits, both are utilized to suggest the "primitive" in the description of the urban core of African American life: "Harlem! Harlem! Little thicker, little darker and noisier and smellier, but Harlem just the same. The niggers done plowed through Hundred and Thirtieth Street" (25). Joyous at his good fortune, Jake wonders where he might have "all this life" if not in "Chocolate Harlem" (14). Drinking, sexuality and sex, enjoyment,music and rhythmic movement are all integral parts of Harlem's charm:

> The deep-dyed color, the thickness, the closeness of it. The noises of Harlem. The sugared laughter. The honey-talk on its streets. And all night long, rag-time and "blues" playing somewhere… singing somewhere, dancing somewhere! Oh, the contagious fever of Harlem. Burning everywhere in dark-eyed Harlem…. Burning now in Jake's sweet blood….(*HH* 15)

All that is Harlem is framed and set against the backdrop of blackness in all of its shades and forms; "colour" is what makes this neighbourhood unique and creates the social group that inhabits it.

## CONCLUSION

McKay emphasizes the distinctions between Black and White culture throughout the novel. In the novel, the author deftly contrasts the free-form, spontaneous, libidinous life of the black ghetto a place where drugs, alcohol, guns, sex, knives, and poverty are commonplace with the puritanical, controlled behaviour of the white society. Jake travels to Baltimore to visit restaurants, taverns, and a cabaret. There, he meets Felice, a young prostitute who is brown, and they spend the night together. Jake then returns to Harlem. For both Black and White audiences, the cabaret is well-known. The police squad's actions, which included promoting and allowing prostitution, pornography, gambling, and the cabaret was forced to close for a long time due to the illicit usage of drugs and alcohol.

McKay's empathy for the issues facing the black working class is evident in the strike-breaking scenes in *Home to Harlem*, which are partially inspired by his personal experiences. McKay worked as a houseman, dining-car waiter, porter, and stevedore before he could support himself from the money he earned from writing. The spirit of his identification with the expanding Black working class is made evident in this proletarian novel. Though it is a "proletarian" novel, McKay's *Home to Harlem* lacks the militant tone of the later proletarian novels of the 1930s. However, McKay honours the vitality and lives of the black working class in Harlem in the novel *Home to Harlem*. These people include stevedores, maids, dockers, cooks, waiters, prostitutes, and pims, who taken as a whole provide a new energy that white society completely slacks.

Thus, the article mainly focuses on the social life of blacks in *Home to Harlem*. Additionally, McKay expertly captures the social life of blacks in Harlem and portrays them as self-assured individuals who are proud of their race. Even though Jake is a social reformer, his self-esteemed nature does not allow him to serve for his nation. So, he escapes from the army and tries to find his color pride in his homeland. Ray feels too proud of his color. The character seems to be the mouthpiece of Claude McKay, in order to voice against racial discrimination and racial prejudice that prevailed in his contemporary society.

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**RESEARCH ARTICLE**

# A Methodology to Propose Neutrosophic Basic Probability Assignment in Neutrosophic Evidence Set

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**ABSTRACT**

Many data fusion application systems make use of the Dempster – Shafer theory of evidence. In evidence theory, determining Basic Probability Assignment is still a work in progress. A method for locating Neutrosophic Basic Probability Assignment in a Neutrosophic set is proposed in this study. Finally, a real-life example demonstrates the cogency of the planned strategy.

**Keywords:** Dempster Shafer concept, Neutrosophic evidence sets, Combination rule, Basic probability assignment.

## INTRODUCTION

Neutosophy is a relatively novel area of attitude. To contract with partial, inconsistent, then uncertain information, FlorentinSmarandachefuture the idea of Neutrosophic circles. The neutral customary is a helpful mathematical tool that is a generalisation of classic sets, conventional fuzzy sets, and intuitionistic fuzzy sets. Each statement in neutrosophic logic has a certainty degree (T), an indeterminancy degree (I), and a falsehood degree (F), where T, I, and F are normal or nonstandard subsets of]-0, 1+ . The Dempster-Shafer Theory is a mathematical evidence theory. One of the most widely used uncertainty theories is the D–S model of indication [2] [12]. The theory of probable or evidential reasoning is another name for it. It is a powerful theoretical tool that may be used to describe partial knowledge and update opinions. The Dempster Shafer model of uncertainty representation and processing offers a wide variety of useful applications, including information fusion[5],[15],[17], expert systems [7], decision making

[8],[9], and risk assignment [4]. Evidence theory relies heavily on basic probability assignment. Basic probability assignment can be used to define all other measures. Expert subjectivity is generally used to provide the BPA in practise. Many other theories, such as fuzzification [20], intuitionistic sets [1], and so on., and so on, have been expanded to cope with uncertainty in addition to the evidence theory. The notion of interval valued evidence[14], R-No's [11], and other methods can be used to enhance how the DS –theory represents unclear data

Another variation on basic probability assignment is offered in this study. Only the truth membership of inference is considered in the classic basic probability assignment. A neutrosophic evidence set is proposed, which is based on the ideas of sets of neutrosophic. The neutrosophic evidence set takes into account the focal components' participation in the truth, indeterminacy, and falsity. Neutrosophic Basic Probability Assignment is an important aspect of the neutrosophic set (NBPA). The first BPA denotes the truth membership, the second BPA denotes the indeterminacy membership, and the third BPA denotes the falsity membership in a Neutrosophic Basic Probability Assignment. Neutrosophic sets have singletons as their fundamental values, however the focal element of Neutrosophic Basic Probability Assignment can be a multiple subset of the discernment frame. The proposed neutrosophic evidences provide a more adaptive technique to model decision - making uncertainty. It combines Dempster – Shafer evidence theory with the capacity to cope with extra information through neutrosophic sets.

The following is a breakdown of the paper's structure: In section 2, the Dempster- Shafer Theory and the neutrosophic set are briefly discussed. We presented Neutrosophic basic probability assignment in Section 3. Section three employs multi-criteria group decision making to illustrate the correctness of the proposed strategy. Finally, in section 5, there are some closing remarks.

**Preliminaries**

**Definition : 2.1** [6]

Consider X to be a collection of points, with x being a generic member in X. The truth membership function, $T_{A.}$, and the indeterminancy membership function, $I_{A.}$, and falsity membership function, $F_{A.}$ characterise a neutrosophic set A in X. $F_{A.}$,,$T_{A.}$,x.,,$I_{A.}$(x), and, $F_{A.}$(x) are real standard or non-standard subsets of ]-0, 1+ [.

That is, $T_A : X \rightarrow ]0^-, 1^+[$ ,
$I_A : X \rightarrow ]0^-, 1^+[$ ,
$F_A : X \rightarrow ]0^-, 1^+[$ .
The sum of $T_A$ (x),$I_A$ (x) and $F_A$(x) has no restriction, so $0^- \leq \sup T_A(x) + \sup I_A(x) + \sup F_A(x) \leq 3^+$.

**Definition : 2.2**[4] ,[12]

Let the frame of discernment $\ominus = \{k_1, k_2, k_3, \dots, k_n\}$. The power set of $\ominus$ is $2^{\ominus} = \{\emptyset, \{k_1\}, \{k_2\}, \dots, \{k_n\}, \{k_1, k_2\}, \dots, \{k_1, k_2, k_3, \dots, k_i\}, \dots, \ominus\}$. A basic probability assignment (BPA) function m is a mapping of $2^{\ominus}$ to a interval [0,1] defined as [4], [14]:

$m: 2^{\ominus} \rightarrow [0,1]$
which satisfies the following conditions:
i) $m(\emptyset) = 0$ and
ii) $\sum_{A \in 2^{\ominus}} m(A) = 1.$
where $\emptyset$ is an empty set, and A is any element of $2^{\ominus}$. The mass m(A) shows the degree of the evidence support A.

**Definition : 2.3**[4] ,[12]

For a BPA m on $\ominus$, each element of $2^{\ominus}$ such that $m(A) > 0$ is called an focal element of m.

**Definition : 2.4**[4] ,[12]
For a BPA m on $\ominus$, the belief function Bel, and the plausibility function Pl are defined by

$$\mathrm{Bel}(A) = \sum_{B \subseteq A} m(B)$$

$$\mathrm{Pl}(A) = \sum_{A \cap B \neq \emptyset} m(B)$$

**Definition : 2.5**[4]
Given two BPAs $m_1$ and $m_2$ on $\ominus$, the rule of combination is defined by

$$m_1 \otimes m_2(\emptyset) = 0$$

$$m_1 \otimes m_2(\emptyset) = \frac{\sum_{B \cap C = A} m_1(B) m_2(C)}{1 - \sum_{B \cap C = \emptyset} m_1(B) m_2(C)}$$

**Definition : 2.6**[3] [10]
Given two BPAs $m_1$ and $m_2$ on $\ominus$, the weights of two BPAs are $w_1$ and $w_2$. A is any subset of $\ominus$.The weighted averagecombination method is defined as

$$m_1 \otimes m_2(A) = \mathrm{ave}\,(m_1, m_2) \otimes \mathrm{ave}\,(m_1, m_2)(A)$$

Where ave $(m_1, m_2)$ is a new BPA and it is defined by

$$\mathrm{ave}\,(m_1, m_2)(A) = m_1(A) \times w_1 + m_2(A) \times w_2$$

**Definition : 2.7**[13]
The Pignistic probability of a BPA m on $\ominus$is defined as

$$\mathrm{Bet}\,P(A) = \sum_{B \in 2^{\ominus}} \frac{|A \cap B|}{|B|} \frac{m(B)}{1 - m(\emptyset)}, \forall A \in 2^{\ominus}$$

where $|A|$ is the cardinality of A. The pignistic probability is a probability distribution on $\ominus$with $p_k = \mathrm{Bet}\,P(h_k) = \sum_{h_k \in B} \frac{m(B)}{|B|}$

**Definition : 2.8** [18]
Assume m is a BPA on $\ominus$, the cost of a focal element$A, A \subseteq \ominus$is defined as

$$\mathrm{Cost}\,(A) = \frac{n - |A|}{n - 1}$$

Assume m is a BPA on $\ominus$, the cost of m is defined as

$$\mathrm{Cost}\,(m) = \sum_{A \subseteq \ominus} \mathrm{Cost}\,(A) m(A)$$

**Definition : 2.9**[16]
Assume m is a BPA on $\ominus$, the specificity of m is defined as

$$\mathrm{Sp}(m) = \sum_{A \subseteq \ominus, A \neq \emptyset} \frac{m(A)}{|A|}$$

**Neutrosophic Basic probability Assignment:**

**Definition: 3.1**
Assume a frame of discernment $\ominus = \{k_1, k_2, k_3, \dots, k_n\}$. The power set of $\ominus$is $2^{\ominus} = \{\emptyset, \{k_1\}, \{k_2\}, \dots, \{k_n\}, \{k_1, k_2\}, \dots, \{k_1, k_2, k_3, \dots, k_i\}, \dots, \ominus\}$. A Neutrosophic basic probability assignment (NBPA) of $2^{\ominus}$ is defined as

$$m(A) = \langle m^T(A), m^I(A), m^F(A) \rangle \quad (1)$$

where $m^T(A): 2^{\ominus} \to [0,1]$ ,$m^I(A): 2^{\ominus} \to [0,1]$ and $m^F(A): 2^{\ominus} \to [0,1]$ are truth membership, indeterminancy membership and falsity membership respectively.

A is any element of $2^{\ominus}$. They must satisfy the following conditions:

i) $m^T(\emptyset) = 0, m^I(\emptyset) = 0$ and $m^F(\emptyset) = 0$.

ii) $\sum_{A \in 2^{\ominus}} m^T(A) = 1$.

iii) For all $A \neq \ominus$, $m^T(A) + m^I(A) + m^F(A) \leq 1$.

**Definition: 3.2**

For Neutrosophic Basic Probability Assignment 'm' on $\ominus$, each subset A of $\ominus$ such that $m^T(A) > 0$ or $m^I(A) > 0$ or $m^F(A) > 0$ is called a focal element of 'm'.

**Definition: 3.3**

Let 'm' be a Neutrosophic Basic Probability Assignment on $\ominus$ with Neutrosophic Probability masses $m(A) = \langle m^T(A), m^I(A), m^F(A) \rangle$ and $A \in 2^{\ominus}$. The Belief function of A also have three components defined as:

$$Bel(A) = < Bel^T(A), Bel^I(A), Bel^F(A) > \quad (2)$$

where $Bel^T(A) = \sum_{B \subseteq A} m^T(B)$ (3)

$$Bel^I(A) = \sum_{B \subseteq A} m^I(B) \quad (4)$$

$$Bel^F(A) = \sum_{B \subseteq A} m^F(B) \quad (5)$$

**Definition: 3.4**

Let 'm' be a Neutrosophic Basic Probability Assignment on $\ominus$ with Neutrosophic Probability masses $m(A) = \langle m^T(A), m^I(A), m^F(A) \rangle$ and $A \in 2^{\ominus}$. The Plausibility function of 'A' also have three components defined as:

$$Pl(A) = < Pl^T(A), Pl^I(A), Pl^F(A) > \quad (6)$$

where $Pl^T(A) = \sum_{B \cap A \neq \emptyset} m^T(B)$ (7)

$$Pl^I(A) = \sum_{B \cap A \neq \emptyset} m^I(B) \quad (8)$$

$$Pl^F(A) = \sum_{B \cap A \neq \emptyset} m^F(B) \quad (9)$$

**Definition: 3.5**

The possibility of a subset 'A' also have three components $Poss(A) = \langle Poss^T(A), Poss^I(A), Poss^F(A) \rangle$(10)

where $Poss^T(A) \in [Bel^T(A), Pl^T(A)]$ (11)

$$Poss^I(A) \in [Bel^I(A), Pl^I(A)] \quad (12)$$

$$Poss^F(A) \in [Bel^F(A), Pl^F(A)] \quad (13)$$

**Definition: 3.6**

Let 'm' be a Neutrosophic Basic Probability Assignment on $\ominus$ with Neutrosophic Probability masses $m(A) = \langle m^T(A), m^I(A), m^F(A) \rangle$ and $A \in 2^{\ominus}$. The pignistic probability of 'm' is defined by

$$Bet\,P(A) = \langle Bet\,P^T(A), Bet\,P^I(A), Bet\,P^F(A) \rangle \text{ for all } A \in 2^{\ominus}. \quad (14)$$

where

$$Bet\,P^T(A) = \sum_{B \in 2^{\ominus}} \frac{|A \cap B|}{|B|} \cdot \frac{m^T(B)}{1 - m^T(\emptyset)} \quad (15)$$

$$Bet\,P^I(A) = \sum_{B \in 2^{\ominus}} \frac{|A \cap B|}{|B|} \cdot \frac{m^T(B)}{1 - m^T(\emptyset)} \quad (16)$$

$$Bet\,P^F(A) = \sum_{B \in 2^{\ominus}} \frac{|A \cap B|}{|B|} \cdot \frac{m^T(B)}{1 - m^T(\emptyset)} \quad (17)$$

**Definition: 3.7**

Let the neutrosophic probability mass of'A', $\langle m^T(A), m^I(A), m^F(A)\rangle$ is an Neutrosophic Basic Probability Assignment'$m'$ on $\ominus$.

The Pure support degree is defined as

$PS(A) = \max(\text{Bet } P^T(A) - \text{Bet } P^I(A), \text{Bet } P^T(A) - \text{Bet } P^F(A))$(27) (18)

**Definition: 3.8**

The cost of a Neutrosophic Basic probability assignment 'm' is defined by

$$\text{cost}(m) = \sum_{A\subseteq\ominus} \text{cost}(A)\, m^T(A) + \sum_{A\subseteq\ominus} \text{cost}(A)\, m^I(A) + \sum_{A\subseteq\ominus} \text{cost}(A)\, m^F(A) \quad (19)$$

When all focal elements of 'm' are singleton, the cost of 'm' is the most cost, cost(m)=3.

When 'm' with one focal element $m(\ominus) = \langle 1,1\rangle$, the cost of 'm' is the least cost, cost(m)=0.

**Definition: 3.9**

The specificity of Neutrosophic probability assignment'm' is defined as

$$sp(m) = \sum_{A\subseteq\ominus, \mathbf{A}\neq\emptyset} \frac{m^T(A)+m^I(A)+m^F(A)}{|A|} \quad (20)$$

**Definition: 3.10**

Assume 'm' is a Neutrosophic Basic Probability Assignment on $\ominus$with neutrosophic probability massesm(A) = $\langle m^T(A), m^I(A), m^F(A)\rangle$ and $A \in 2^{\ominus}$. Then $m^*$ is a Basic probability transformed by 'm' can be calculated by

$$m^*(A) = \min\left[\frac{m^T(A)-m^I(A)}{\sum_{B\subseteq\ominus}(m^T(B)-m^I(B))}, \frac{m^T(A)-m^F(A)}{\sum_{B\subseteq\ominus}(m^T(B)-m^F(B))}\right] \quad (21)$$

**Definition : 3.11**

Given two Neutrosophic Basic Probability Assignment'$m_1$' and '$m_2$' on $\ominus$, the combination result is denoted as$m_1 \otimes m_2$is given by

$m_1 \otimes m_2(A) = \langle m^T(A), m^I(A), m^F(A)\rangle$ (22)

where

$$\begin{cases} m^T(\emptyset) = 0 \\ m^T(A) = \dfrac{\sum_{B\cap C=A} m_1^T(B) m_2^T(C)}{1 - \sum_{B\cap C=\emptyset} m_1^T(B) m_2^T(C)} \end{cases} \quad (23)$$

$$\begin{cases} m^I(\emptyset) = 0 \\ m^I(A) = \dfrac{\sum_{B\cap C=A} m_1^I(B) m_2^I(C)}{1 - \sum_{B\cap C=\emptyset} m_1^I(B) m_2^I(C)} \end{cases} \quad (24)$$

$$\begin{cases} m^F(\emptyset) = 0 \\ m^F(A) = \dfrac{\sum_{B\cap C=A} m_1^F(B) m_2^F(C)}{1 - \sum_{B\cap C=\emptyset} m_1^F(B) m_2^F(C)} \end{cases} \quad (25)$$

**Definition: 3.12**

Let '$m_1$' and '$m_2$'be two Neutrosophic Basic Probability Assignmenton $\ominus$. Then the weighted average combination result NBPA,

$m_1 \otimes m_2(A) = \langle m^T(A), m^I(A), m^F(A)\rangle$is given by

$m^T(A) = \text{ave}\,(m_1^T, m_2^T)(A)$

$m^I(A) = \text{ave}\,(m_1^I, m_2^I)(A)$

$m^F(A) = \text{ave}\,(m_1^F, m_2^F)(A)$

**Illustration of the Proposed Approach:**

Consider the challenge of selecting refrigerator brands. If there are four refrigerator brands, R-1,.,R-2,,R-3, and,R-4, then the alternate set is R=,,R-1,.,R-2,,R-3.,,R-4... A decision group of three decision makers has been constituted to investigate alternative refrigerator brands. D=,,D-1.,,D-2.,,D-3... is the collection of decision makers. Assume the selection problem involves four criteria: C-1. (quality), C-2. (price), C-3. (degree of satisfaction), and C-4. (function).

C=,,C-1,.,C-2,,C-3,,C-4... is the collection of criteria. The steps in the procedure for the selection problem are as follows.

Step : 1
Determine the weights of criteria:
Opinions of all criteria by decision makers are given in the below table :

| $D_1$ | $D_2$ | $D_3$ |
|---|---|---|
| $\{C_1\}\langle 0.3, 0.2, 0.1\rangle$<br>$\{C_2\}\langle 0.2, 0.2, 0.2\rangle$<br>$\{C_3\}\langle 0.2, 0.3, 0.3\rangle$<br>$\{C_4\}\langle 0.2, 0.2, 0.3\rangle$<br>$\{C_1, C_4\}\langle 0.1, 0.1, 0.1\rangle$ | $\{C_1\}\langle 0.2, 0.1, 0.2\rangle$<br>$\{C_2\}\langle 0.2, 0.3, 0.2\rangle$<br>$\{C_3\}\langle 0.2, 0.2, 0.2\rangle$<br>$\{C_4\}\langle 0.1, 0.2, 0.3\rangle$<br>$\{C_1, C_2, C_3\}\langle 0.1, 0.2, 0.1\rangle$<br>$\{C_2, C_3, C_4\}\langle 0.2, 0, 0\rangle$ | $\{C_1\}\langle 0.3, 0.2, 0.1\rangle$<br>$\{C_2\}\langle 0.3, 0.1, 0.1\rangle$<br>$\{C_3\}\langle 0.1, 0.2, 0.2\rangle$<br>$\{C_4\}\langle 0.1, 0.1, 0.2\rangle$<br>$\{C_1, C_4\}\langle 0.1, 0, 0.2\rangle$<br>$\{C_2, C_3\}\langle 0.1, 0.2, 0\rangle$<br>$\{C_2, C_3, C_4\}\langle 0, 0.2, 0.2\rangle$ |

The fused result $m_c$ of decision makers opinions can be calculated by the Equations (22)-(25)

$$m_c(\{c_1\}) = \langle 0.4590, 0.1333, 0.0698\rangle$$
$$m_c(\{c_2\}) = \langle 0.2308, 0.250, 0.1667\rangle$$
$$m_c(\{c_3\}) = \langle 0.2162, 0.4286, 0.3443\rangle$$
$$m_c(\{c_4\}) = \langle 0.1428, 0.1071, 0.4030\rangle$$

The weight of criteria $W_c = [W_{c_1}, W_{c_2}, W_{c_3}, W_{c_4}]$ can be calculated by

$$W_{c_j} = \langle W_{c_j}^T, W_{c_j}^I, W_{c_j}^F\rangle$$
$$= \langle \text{Bet } P^T(c_j), \text{Bet } P^I(c_j), \text{Bet } P^F(c_j)\rangle$$

where $W_{c_j}^T = \text{Bet } P^T(c_j), W_{c_j}^I = \text{Bet } P^I(c_j)$, and $W_{c_j}^F = \text{Bet } P^F(c_j)$ , $j = 1,2,3, \ldots$

Therefore,

$$W_c = \begin{bmatrix} \langle 0.4590, 0.1333, 0.0698\rangle, \langle 0.2308, 0.2500, 0.1667\rangle, \\ \langle 0.2162, 0.4286, 0.3443\rangle, \langle 0.1428, 0.1071, 0.4030\rangle \end{bmatrix}$$

Step : 2
Calculate the initial fused results NBPAs based on weight of criteria.
The NBPA decision matrix M is

$$M = \begin{bmatrix} m_{11} & m_{12} & m_{13} \\ m_{21} & m_{22} & m_{23} \\ m_{31} & m_{32} & m_{33} \\ m_{41} & m_{42} & m_{43} \end{bmatrix}$$

Where,m-jk. is an NBPA allocated by policymakers. to criteria,C-j.
The NBPA of the decision problem is shown in the table below:

| Criteria | Subsets of brands | Decision maker | | |
|---|---|---|---|---|
| | | $D_1$ | $D_2$ | $D_3$ |
| $C_1$ | $\{R_1\}$ | $\langle 0.22, 0.21, 0.23\rangle$ | $\langle 0.22, 0.18, 0.20\rangle$ | $\langle 0.21, 0.30, 0.33\rangle$ |
| | $\{R_2\}$ | $\langle 0.18, 0.13, 0.15\rangle$ | $\langle 0.19, 0.17, 0.14\rangle$ | $\langle 0.15, 0.14, 0.17\rangle$ |
| | $\{R_3\}$ | $\langle 0.27, 0.17, 0.08\rangle$ | $\langle 0.23, 0.21, 0.11\rangle$ | $\langle 0.25, 0.10, 0.08\rangle$ |
| | $\{R_4\}$ | $\langle 0.18, 0.21, 0.23\rangle$ | $\langle 0.19, 0.17, 0.22\rangle$ | $\langle 0.21, 0.15, 0.17\rangle$ |
| | $\{R_1, R_2\}$ | $\langle 0.00, 0.28, 0.31\rangle$ | $\langle 0.00, 0.12, 0.33\rangle$ | $\langle 0.00, 0.23, 0.25\rangle$ |
| | $\{R_1, R_3\}$ | $\langle 0.15, 0.01, 0.00\rangle$ | $\langle 0.17, 0.15, 0.00\rangle$ | $\langle 0.18, 0.08, 0.00\rangle$ |
| $C_2$ | $\{R_1\}$ | $\langle 0.21, 0.20, 0.23\rangle$ | $\langle 0.23, 0.18, 0.20\rangle$ | $\langle 0.19, 0.17, 0.15\rangle$ |
| | $\{R_2\}$ | $\langle 0.18, 0.17, 0.19\rangle$ | $\langle 0.20, 0.11, 0.13\rangle$ | $\langle 0.22, 0.20, 0.23\rangle$ |
| | $\{R_3\}$ | $\langle 0.29, 0.28, 0.05\rangle$ | $\langle 0.23, 0.11, 0.13\rangle$ | $\langle 0.25, 0.23, 0.08\rangle$ |
| | $\{R_4\}$ | $\langle 0.18, 0.17, 0.24\rangle$ | $\langle 0.17, 0.25, 0.27\rangle$ | $\langle 0.19, 0.17, 0.23\rangle$ |
| | $\{R_1, R_2\}$ | $\langle 0.00, 0.06, 0.29\rangle$ | $\langle 0.00, 0.25, 0.27\rangle$ | $\langle 0.00, 0.10, 0.31\rangle$ |
| | $\{R_1, R_3\}$ | $\langle 0.14, 0.12, 0.00\rangle$ | $\langle 0.17, 0.10, 0.00\rangle$ | $\langle 0.15, 0.13, 0.00\rangle$ |
| $C_3$ | $\{R_1\}$ | $\langle 0.21, 0.26, 0.29\rangle$ | $\langle 0.20, 0.28, 0.30\rangle$ | $\langle 0.24, 0.23, 0.27\rangle$ |
| | $\{R_2\}$ | $\langle 0.18, 0.11, 0.14\rangle$ | $\langle 0.17, 0.18, 0.20\rangle$ | $\langle 0.18, 0.07, 0.09\rangle$ |

| | | | | |
|---|---|---|---|---|
| | $\{R_3\}$ | $\langle 0.22, 0.11, 0.14\rangle$ | $\langle 0.23, 0.08, 0.10\rangle$ | $\langle 0.20, 0.16, 0.18\rangle$ |
| | $\{R_4\}$ | $\langle 0.21, 0.12, 0.14\rangle$ | $\langle 0.20, 0.18, 0.20\rangle$ | $\langle 0.20, 0.16, 0.18\rangle$ |
| | $\{R_1, R_2\}$ | $\langle 0.00, 0.27, 0.29\rangle$ | $\langle 0.00, 0.18, 0.20\rangle$ | $\langle 0.00, 0.26, 0.28\rangle$ |
| | $\{R_1, R_3\}$ | $\langle 0.18, 0.13, 0.00\rangle$ | $\langle 0.20, 0.10, 0.00\rangle$ | $\langle 0.18, 0.12, 0.00\rangle$ |
| $C_4$ | $\{R_1\}$ | $\langle 0.22, 0.20, 0.25\rangle$ | $\langle 0.22, 0.27, 0.31\rangle$ | $\langle 0.23, 0.21, 0.20\rangle$ |
| | $\{R_2\}$ | $\langle 0.18, 0.12, 0.17\rangle$ | $\langle 0.15, 0.11, 0.13\rangle$ | $\langle 0.19, 0.17, 0.13\rangle$ |
| | $\{R_3\}$ | $\langle 0.24, 0.03, 0.08\rangle$ | $\langle 0.25, 0.06, 0.08\rangle$ | $\langle 0.23, 0.21, 0.20\rangle$ |
| | $\{R_4\}$ | $\langle 0.21, 0.12, 0.17\rangle$ | $\langle 0.19, 0.21, 0.23\rangle$ | $\langle 0.19, 0.17, 0.20\rangle$ |
| | $\{R_1, R_2\}$ | $\langle 0.00, 0.30, 0.33\rangle$ | $\langle 0.00, 0.22, 0.23\rangle$ | $\langle 0.00, 0.10, 0.27\rangle$ |
| | $\{R_1, R_3\}$ | $\langle 0.15, 0.23, 0.00\rangle$ | $\langle 0.19, 0.11, 0.00\rangle$ | $\langle 0.16, 0.14, 0.00\rangle$ |

The initial fused results $M^{ne} = [m_1^{ne}, m_2^{ne}, m_3^{ne}]$ can be calculated by

$$\text{ave}(m_k^{ne}) = \sum_{j=1}^{4} m_{jk} \times w_{cj}, k = 1,2,3$$

$$m_k^{ne} = \text{ave}(m_k^{ne}) \otimes \text{ave}(m_k^{ne}) \otimes \text{ave}(m_k^{ne}), k = 1,2,3$$

We have obtain

$m_1^{ne}$: $m_1^{ne}(\{R_1\}) = \langle 0.2263, 0.2107, 0.2550\rangle$

$m_1^{ne}(\{R_2\}) = \langle 0.1888, 0.1198, 0.1588\rangle$

$m_1^{ne}(\{R_3\}) = \langle 0.2727, 0.1570, 0.944\rangle$

$m_1^{ne}(\{R_4\}) = \langle 0.1995, 0.1342, 0.1728\rangle$

$m_1^{ne}(\{R_1, R_2\}) = \langle 0, 0.2000, 0.3027\rangle$

$m_1^{ne}(\{R_1, R_3\}) = \langle 0.1615, 0.1249, 0\rangle$

$m_2^{ne}$: $m_2^{ne}(\{R_1\}) = \langle 0.2285, 0.2178, 0.2754\rangle$

$m_2^{ne}(\{R_2\}) = \langle 0.1916, 0.1389, 0.1528\rangle$

$m_2^{ne}(\{R_3\}) = \langle 0.2441, 0.0962, 0.0960\rangle$

$m_2^{ne}(\{R_4\}) = \langle 0.1967, 0.1848, 0.2220\rangle$

$m_2^{ne}(\{R_1, R_2\}) = \langle 0, 0.1792, 0.2296\rangle$

$m_2^{ne}(\{R_1, R_3\}) = \langle 0.1875, 0.0997, 0\rangle$

$m_3^{ne}$: $m_3^{ne}(\{R_1\}) = \langle 0.2250, 0.2033, 0.2216\rangle$

$m_3^{ne}(\{R_2\}) = \langle 0.1857, 0.1168, 0.1335\rangle$

$m_3^{ne}(\{R_3\}) = \langle 0.2485, 0.1619, 0.1614\rangle$

$m_3^{ne}(\{R_4\}) = \langle 0.2106, 0.1492, 0.1927\rangle$

$m_3^{ne}(\{R_1, R_2\}) = \langle 0, 0.1778, 0.2744\rangle$

$m_3^{ne}(\{R_1, R_3\}) = \langle 0.1789, 0.1094, 0\rangle$

Step : 3

Influence the exact fused results:

The NBPA,m-fn. final fused findings are determined by

$m_d$ : $m_d(\{D_1\}) = \langle 0.4, 0.1, 0.2\rangle$

$m_d(\{D_2\}) = \langle 0.2, 0.3, 0.35\rangle$

$m_d(\{D_3\}) = \langle 0.3, 02, 0.25\rangle$

$m_d(\{D_1, D_3\}) = \langle 0.1, 02, 0.1\rangle$

$m_d(\{D_2, D_3\}) = \langle 0, 0.2, 0.1\rangle$

Step : 4 Influence the exact fused results:

The NBPA,m-fn. final fused findings are determined by

$$\text{ave}\left(m^{fn}\right) = \sum_{k=1}^{3} m_k^{fn} \times W_{d_1}$$

$$m^{fn} = \text{ave}\left(m^{fn}\right) \otimes \text{ave}\left(m^{fn}\right) \otimes \text{ave}\left(m^{fn}\right), k = 1,2,3.$$

Hence

$m^{fn}$ : $m^{fn}(\{R_1\}) = \langle 0.2263, 0.2105, 0.2515\rangle$

$m^{fn}(\{R_2\}) = \langle 0.1883, 0.1261, 0.1475\rangle$

$m^{fn}(\{R_3\}) = \langle 0.2564, 0.1347, 0.1185\rangle$

$m^{fn}(\{R_4\}) = \langle 0.2027, 0.1604, 0.1994\rangle$

$m^{fn}(\{R_1, R_2\}) = \langle 0, 0.1828, 0.2635 \rangle$

$$m^{fn}(\{R_1, R_3\}) = \langle 0.1102, 0.1086, 0 \rangle$$
$$m^{fn}(\ominus) = \langle 0.0140, 0.0769, 0.0196 \rangle$$

Step : 5

Rank alternatives:

(14)-calculates the simple endorsement degree of every option in,m-fn (18). Then, in order of pure support degrees, rank the options as follows.

$$PS(R_1) = -0.0452$$
$$PS(R_2) = -0.0198$$
$$PS(R_3) = 0.1748$$
$$PS(R_4) = 0.0109$$

The alternatives were ranked as $R_3 > R_4 > R_2 > R_1$.

Therefore, among the four refrigerators brands$R_3$ should be selected.

## CONCLUSION

In the application of DS theory to complicated uncertain circumstances, BPA estimate is quite significant. Unlike standard BPA, the proposed NBPA takes into account the truth, indeterminacy, and falsity membership of focal elements. This technique combines the strengths of evidence theory and the Neutrosophic set, making it more flexible, information-rich, and reasonable. Unlike the Neutrosophic set, the proposed NBPA allows numerous subsets of the frame of discernment to be members of truth, indeterminacy, and falsehood. An example of group decision making is used to show the effectiveness of the proposed technique.This approach is used to choose the best refrigerator brands based on four criteria: quality, price, level of satisfaction, and functionality.

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**RESEARCH ARTICLE**

# Detergent Activity of Aqueous Extract of Croton *Bonplandianus* Baill - Possible Source of Natural Surfactant

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## ABSTRACT

*Croton bonplandianus* Baill is a perennial herb grows in all climatic conditions in wasteland areas. The extract of these plants contains several secondary metabolites used as medicine for various diseases. The phytochemical analysis of this plant shown to contain high amount of saponins that act as surfactant revealed an excellent detergent activity. This study focused to determine the detergent activity of *Croton bonplandianus* Baill using various parameters such as preliminary phytochemical analysis, dirt dispersion, cleaning activity, foaming stability, pH and wetting ability. This plant possesses high dirt dispersion and cleaning activity compared to SLS standard. The foaming stability is less compared to standard but wetting ability show maximum in plant extract in 1% as well as 5%. The pH of drug sample is found to 6.11 which is essential factor for detergent quality. The foam and froth was made to stand for maximum time to reflect the presence of saponins. Further HPLC analysis confirmed the presence of saponin with retention time of 2.684 min similar to standard.

**Keywords:** *Croton bonplandianus* Baill, Saponin, SLS, Surfactant, HPLC

## INTRODUCTION

A chemical compound with the ability to clean is said to be a detergent. An excellent detergent dissolves in water, lowers the interfacial tension to allow the water-based solution to pass through the capillaries, disperses clumped particles, and bonds the dirt or particles of oil to the water instead of the material being cleaned (Haney *et al*., 1954). Many secondary chemicals generated by plants are bio-based surfactants, which resemble soap or detergent (Wisetkomolmat *et al*., 2019). In aqueous solutions, detergents are amphipathic compounds that are surface-active agents – they display special characteristics, such as the ability to develop spherical micellar structures and reduce the tension on the surface of their solution. The primary objective of detergents is to eliminate stains, which are typically caused by protein bases, oils, and other contaminants without causing any harm or re-depositing dirt onto the material (Bhat *et al*., 2011). Many medicinal plants contain natural surfactants such as saponin which can form stable soap-like foam when shaken in water (Wisetkomolmat *et al*., 2021). The desire to substitute chemical synthesis that harms our environment has increased demand for these important active components.

Asian developing nations continue to rely heavily on medicinal herbs in their daily lives. As a result, these plants are essential for daily existence. Flavonoids, tannins, steroids, alkaloids, saponins, terpenoids, cardiac glycosides, flavonoids, anthraquinone and reducing sugars are the primary phytochemicals found in plants (Agidew, 2022).Saponins are important plant glycosides among secondary metabolites that are widelydistributed in the plant kingdom and it ispresent in herbs, vegetables, and beans. Moreover, saponins are amphipathic combinations of hydrophobic aglycone and hydrophilic sugar molecules that makes strong surface-active compounds (Samal et al., 2017; Kregiel et al., 2017). Hence, it possesses foaming, detergent, emulsifying and wetting properties. Previous research report stated that saponins were identified from the leaves of *Mangifera indica* L., juice of citrus fruit,leaves and roots of *Leucas aspera* L., fruits of *Persea americana*, leaves of *Vernonia amygdalina* bark of *Vernonia amygdalina*(Agidew, 2022).

The monoecious exotic weed, *Croton bonplandianus* Baill, commonly referred as Ban Tulsi, is typically 30–40 cm tallthat grows along roadside ditches, wide open ravines fields of railway, and agricultural fields. Report says that it is native to India, Bangladesh, South Western Brazil, South America, North Argentina, and Pakistan. The plant parts and latex used for treatment of wounds, treatment for skin diseases, possesses antiseptic and antimicrobial activity (Ghosh *et al*., 2018).It was reported that methanolic extract of *Croton bonplandianus* Baill revealed the presence of resins, alkaloids, saponins, flavonoids, saponins, phenols, and steroids to shown to contain the pharmacological activity (Jeeshna *et al*, 2011). Saponin and flavonoid contents are maximum compare to other metabolites. Hence, this study is focused on the detergent activity of aqueous extract of *Croton bonplandianus* Baill.

## MATERIALS AND METHODS

**Collection and Identification of Plant Samples**

The plants samples were collected from a farm in Sulur, Coimbatore and it was identified by Botanical Survey of India (BSI), Tamilnadu Agriculture University (TNAU), Coimbatore, Tamilnaduas *Croton bonplandianus* Baill belonging to Euphorbiaceae family with Voucher number BSI/SRC/5/23/2023/Tech-186. A specimen of the plant was also deposited for the herbarium.

**Extraction of Sample**

The leaves of the plant were cleaned and shade dried for 72 h.It was powdered, sieved and used for extract preparation. 60 g of the powder was macerated with 600 ml of sterile distilled water for 24 h in a glass stoppered conical flask at 37°,shaken for 6 h and allowed to stand for 18 h, filtered and stored in airtight container for further use (Manzoor *et al*., 2021).

**Preliminary qualitative phytochemical analysis**

Following conventional methods, the primary groups of phytochemicals present in the extract were identified using standard procedure (Lawal *et al*., 2019; Dubale *et al*., 2023).

**Dispersion of Dirt**

The main purpose of a detergent is to clean filth. In test tubes containing 10 ml of distilled water, the extract was added at a concentration of 1% and 5%. The tubes were shaken well and 1 drop of Indian ink was added to the tubes. The tubes were mixed vigorously and allowed to stand for few seconds. The amount of ink present in the foam was estimated as heavy, moderate, light or none (Madhusudhan*et al*.,2021; Manzoor *et al*., 2021).

**Cleaning Action**

The ability of cleaning of a detergent is determined using heavy stains such as greasy cotton cloth test followed by Barnett and Powers (1951). The cleaning ability of detergent is to reduce the surface tension of water and emulsify either grease or oil. Around 5gm of grease was applied on a clean cotton cloth, weighed and placed in tubes containing 1% and 5% of leaf and maintained at 35° for 4 min using a magnetic stirrer with hot plate. The cloth was then taken out, dried for 6 h and measured the weight (Manzoor *et al*., 2021). The following equation was used to calculate the percentage reduction of the grease;

$$DP = 100(1-T/C)$$

Where, DP = Percentage of detergency power; C = Weight of of the sample before cleaning; T = Weight of the sample after cleaning.

**Foaming power and Foaming Stability:**

The cylinder shake method was used to determine the foaming ability. Each 50 ml of 1% and 5% of extract solution were taken in graduated cylinder and shook for 10 times. The total volume of foam was measured at 0 and 5 minutes. By using the foam volume, foam stability was calculated by using R5 parameter which represents the quotient of the foam (Manzoor *et al*., 2021). The residual foam ratio was determined by using the following formula;

$$R_5 = (h_5/h_0) \times 100$$

Where, $h_0$ represents foam height at 0 minute; $h_5$ represents foam height after 5 minutes

**Wetting Ability**

The wetting ability of plant extract was used to determine the efficiency of presence of surfactants. 1 inch diameter of cotton cloth with average weight of 0.32 gm was floated on the surface of the extract. The time taken to sink was observed to determine the wetting ability (Manzoor *et al*., 2021).

**Determination of pH**

The pH of the extract is an essential factor to check the quality of detergent which will minimize the irritation and ecological stability of the skin. The pH of 1% as well as 5% sample extracts was determined thrice with help of digital pH meter at 25°.

**Qualitative test for Saponins**

**Froth test**

The aqueous extracts of the sample and 1% and 5% of SLS (Sodium Lauryl Sulfate) as standard wereshaken vigorously shaken and stand for 10 minutes. The froth of more than 1.5cm indicated the presence of saponins, whereas no froth indicated the absence of saponins.

**Foam test**

Around 2 ml of extract was mixed with 6 ml of distilled water, vigorously shaken for 5 minutes and observed the foam formation. The formation and persistence of foam indicated the presence of saponins.

**HPLC Analysis of Saponins**

About 2 ml of plant extract was dissolved in HPLC grade methanol for HPLC analysis using saponin standard. Shimadzu lab Chromo 2010 HT HPLC, UV detector was used for analysis using a C18 column at 35° temperature. 40: 60 ratio of acetonitrile: water was used as mobile phase; 15µl injection volume for 25min at flow rate 1ml/min at 203nm.

## RESULTS AND DISCUSSION

**Collection, Identification and Extraction of Plant Sample**

The leaves of the collected plant was extracted using sterile water and were used for the phytochemical analysis.

**Preliminary qualitative phytochemical analysis**

From the phytochemical analysis, the identified plant *Croton Bonplandianus* Baill contains high amount of saponins and flavonoids (Table 1). Saponins are the main component that exhibits detergent activity (Rai *et al*., 2021). The plant containssaponinsat a high concentration,which can be utilized for preparation of cleansing agent for both household as well as cosmetic industry. To confirm the potential of the prepared plant extract for cleaning action, further tests were carried out.

**Dispersion of Dirt and Cleaning Action:**

Dirt dispersion activity is estimated to check the ability of the extract to remove the dirt from the test surface by the principle of dispersion. For this, the component must have less frothing ability (Pradhan *et al*., 2022). It can be understood that since the extract produces less foam, the dirt that binds with detergent gets dispersed on the surface of the water, which makes it easy to wash off (Akbari *et al*., 2023). When the foam is high, the dirt gets bound to the foam and will not disperse. If the test surface is immersed in water, the dirt redeposits on the surface again making it impossible to remove. From Table 2, the extract has a good dirt dispersion activity compared to the standard SLS. Similarly, the grease reduction percentage is found higher than SLS (Figure 1). Hence, the plant under study has a good detergent activity at 5% concentration compared to 1%.

**Foaming power and Foaming Stability:**

The above results (Table 3) show less foam formation compared to the standard and the foam has less stability than SLS which indicates its effective cleansing ability (Amagliani *et al*., 2021).

**Wetting Ability**

Wetting ability is an indicator that determines the extent of the detergent's interaction with water. This factor is important because, the detergent when interacting with water starts forming micelles in the absence of water which traps the dirt and releases when immersed in water. This process enables the dirt to get dispersed in water and get washed (Rekiel*et al*., 2020). Moreover, the detergent will be in suspension in water enabling more surface area to be produced. This process helps in trapping of more dirt particles and hence enhanced cleansing activity. The extract under study has an effective wetting ability at 5% concentration. The time taken for sinking as suspension is quicker at 1% compared to 5% (Table 4).

**Determination of pH**

The average pH of the drug solution was found as 6.11 in 1% and 5% of extract (Schreiner *et al*., 2021).

**Qualitative test for Saponins**

**Froth test and Foam test**

The aqueous extracts of the sample showeda froth of 1.5cm and standing timeof 10 min. Foam was observed in aqueous extract after diluting with distilled water. It was an evidence for the presence of saponins. The foam formed was moderate and the standing time indicated the foam does not retain for a longer time. Less the standing time, easy the washing process and water consumption will be less (Góral and Wojciechowski, 2020).

**HPLC Analysis of saponins**

The HPLC analysis of standard hada retention time of 2.315 minutes. The peak of plant extract confirmed the presence of saponin with retention time of 2.684 minutes (Figure 2). This preliminary analysis showed the presence of saponin in *Croton bonplandianus* Baill.Further analysis is needed to extract and purify the exact saponin component and estimate its efficiency (Wang *et al*., 2021).

**CONCLUSION**

The study indicates a novel plant whose extract showed detergent activity. The results obtained also confirms the findings that *Croton bonplandianus* Baill can be utilized for preparing cleansers. Natural cleansers are always preferred compared to synthetic and semisynthetic ones due to their prolonged negative effects or delayed hypersensitive reactions. Extensive research on this plant can give novel phytochemical which could have a wide industrial application.

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**Table 1: Preliminary qualitative phytochemical analysis**

| S.No. | Phytochemical tests | Observation |
|---|---|---|
| 1. | Cardiac Glycoside test | + |
| 2. | Alkaloid test | _ |
| 3. | Saponin test | ++ |
| 4. | Tannin test | + |
| 5. | Carbohydrate test | + |
| 6. | Phytosterol test | _ |
| 7. | Flavonoids test | ++ |
| 8. | Phenol/tannins | _ |

**Table 2: Dirt Dispersion and Cleaning Ability of Test Drug**

| S. No. | Drug Name | Dirt Dispersion | | % of Grease Reduction | |
|---|---|---|---|---|---|
| | | 1% | 5% | 1% | 5% |
| 1 | Distilled water | - | - | 0 | 0 |
| 2 | SLS | Light | Light (+) | 100.3 | 118 |
| 3 | *C. bonplandianus* | Light | Light (+) | 108.7 | 136.1 |

**Table 3: Effect of test drugs on foaming stability**

| S. No. | Drug Name | Foaming Power | | | | Foaming Stability(R5) | |
|---|---|---|---|---|---|---|---|
| | | 1% | | 5% | | | |
| | | 0 min | 5 min | 0 min | 5 min | 1% | 5% |
| 1 | SLS | 48.00 | 130.00 | 42.00 | 192.00 | 270.8 | 457.1 |
| 2 | *C. bonplandianus* | 46.00 | 98.00 | 40.00 | 110.00 | 213.0 | 275.0 |

**Table 4: Effect of test drug on wetting ability**

| S.No. | Drug Name | 1% | | 5% | |
|---|---|---|---|---|---|
| | | Sinking time (hh.mm.ss) | Wetting time (hh.mm.ss) | Sinking time (hh.mm.ss) | Wetting time (hh.mm.ss) |
| 1. | Distilled water | 54.54.00 | 02.52.00 | 54.55.00 | 02.52.00 |
| 2. | SLS | 15.43.00 | 43.50.00 | 04.09.00 | 06.52.00 |
| 3. | *C. bonplandianus* | 01.01.27 | 02.20.10 | 04.10.00 | 17.12.00 |

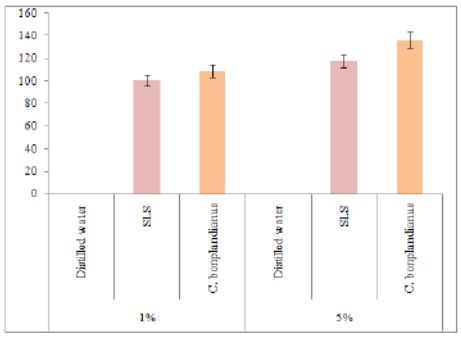


**Figure 1: Percentage grease reduction of the extract**

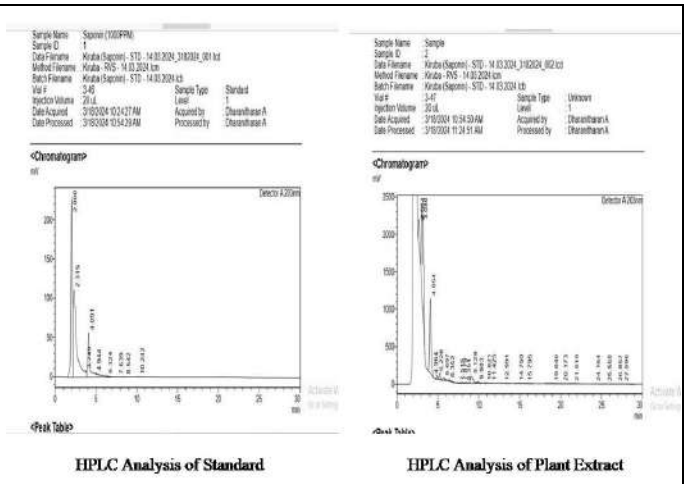


**Figure 2: HPLC Analysis of Saponin**

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**RESEARCH ARTICLE**

# Isolation of Bioactive Compounds from *Streptomyces griseus* DR38

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## ABSTRACT

Actinomycetes are one of the major sources of bioactive compounds that are used against antibacterial, antifungal, antiviral, anticancer and anti-inflammatory based drug discovery in pharmaceutical industry. *Streptomyces* is the largest genus of actinomycetes which is known for the production of most antibiotics. Present study was aimed to isolate the novel actinomycetes from marine sediment. Using crude ethyl acetate extract of actinomycetes antimicrobial screening was performed against various bacterial pathogens such as *E.coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus*and *Streptococcus sp.* in well diffusion method. The strain which had themaximum zone of inhibitionwas selected for further studies and it was identifed as *Streptomyces griseus DR38* through 16S rRNAsequencing.The bioactive compounds have identified using GCMS study.The major active extracellular compound such as D-xylose, Rutinose, Diethyl Phthalate, Phthalic acid, 1,2 Benzenedicarboxylic acid was found in this study. These compounds have higher applications in pharmaceutical, cosmetic and food industry.

**Keywords:** Actinomycetes, Bioactive compounds, Antibiotics, GCMS, *Streptomyces*.

## INTRODUCTION

Actinomycetes, particularly marine actinomycetes are prolific source of bioactive compounds with diverse biological activities such as antibacterial, antifungal, antiviral, and anticancer properties. Studies have highlighted the isolation of numerous bioactive compounds from actinomycetes, including terpenes, terpenoids, meroterpenoids, polyketides, non-ribosomal peptides and other cytotoxic compounds, showcasing their potential in drug discovery and antibiotic development [1],[2]. Actinomycetes from various environments like marine sediments, hot springs and terrestrial ecosystems have been explored for their ability to produce novel bioactive compounds, emphasizing the importance of these microorganisms in generating new sources of pharmaceutical agents. The bioactive compounds isolated from actinomycetes have shown promising activities against drug resistant pathogens, making them valuable candidates for the development of new antibiotics and therapeutic agents [3],[4]. Streptomyces strains have been identified as valuable sources of specialized metabolites, including pigments, which have various bioactivities such as antioxidant, antibiofilm and antibiotic activities. Present study has isolated streptomycesgriseus from marine sediment soil and finding the active compounds through GCMS. These studies highlight the diverse range of bioactive compounds for potential pharmaceutical and nutritional applications.

## MATERIALS AND METHODS

**Sample Collection**

Mangrove soil originating from a specific site in India was procured for research purposes. The soil sample was collected from Pichavaram Mangrove National Forest in Killai, Tamil Nadu India. The collection process involved obtaining soil specimens from a depth of 15 centimetres beneath the soil's surface, which were subsequently placed in aseptic containers for transportation to the laboratory. The process of isolating Actinomycetes from the Mangrove soil entailed utilizing a Starch Casein Agar (SCA) medium comprising of a mixture of 50% seawater and 50% distilled water.

**Screening for Actinomycetes that Produce Bioactive Compounds**

The strains that were isolated underwent screening to assess their capability in producing bioactive compounds. These isolated actinomycetes strains were introduced into a medium containing 50% seawater known as (SCA) medium. The flask containing the inoculated strains was placed in an incubator at room temperature for duration of 5 days while being agitated on a rotary shaker operating at 120 rpm and a temperature of 28$^{o}$C. Following the completion of the incubation period, the liquid broth was filtered, and the resulting filtrate was utilized to evaluate antimicrobial activity.

**Antimicrobial activity**

Antimicrobial efficacy was evaluated using well susceptibility methods, with crude ethyl acetate extracts of active producing strains tested against various microorganisms including *E.coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus*and *Streptococcus sp.* using Muller Hiton Agar Medium (Himedia, India) [5].The strain which had the maximum zone of inhibition was used for further studies.

**Molecular Characterization**

The genomic DNA was isolated using HiPureA Bacterial Genomic DNA Purification kit (Himedia). Primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-TACGGCTACCTTGTTACGACTT -3′) were used to amplify the 1480 bp 16S rRNA. The following were the PCR (Prime96 – Himedia) conditions: Initial denaturation 94$^{o}$C for 3min, followed by 30 cycle of denaturation at 94$^{o}$C for 1min, annealing at 55$^{o}$C for 1min, extension at 72$^{o}$C for 1 min, and final extension at 72$^{o}$C for 3 min. The amplified product was confirmed with 1.2% of agarose gel. The PCR products were sequenced with Sanger Sequencing 3730xl DNA Analyzer (Barcode BioSciences – Bengaluru). The 16S rRNA sequence was entered into the NCBI nucleotide blast to determine the identity and similarity of the sequence with

other species in the database. Mega 11 software was used for sequence alignment and generation of phylogenetic trees.

**Identification of Bioactive compounds with GCMS**

The analysis of the active ethyl acetate extract was executed by Gas Chromatography Mass Spectrometry (GCMS). Shimadzu QP2010 ultra was used for identification of compounds and gas chromatography interfaced to a mass spectrometer. The appliance was built with Elite-1 fused silica capillary. Helium gas (99.99%) was the carrier gas with a constant flow rate of 1.21 ml/min and split ratio:10. Temperature of Injector was set on 160oC; Ion-source temperature was 200oC. The oven temperature was intended from 60$^{o}$C with an increment of 280$^{o}$C for around 22 min. Mass spectra were taken at 70eV; a scan interval of 0.5 sc. The chemical composition of the extract was determined by measuring the peak area and the retention time by comparing the NIST 14 library [6],[7].

## RESULT AND DISCUSSION

Primary and secondary screening of strain was performed against bacterial pathogens in well diffusion method was applied on Muller Hinton Agar Plates[6]. The maximum zone of inhibition in *Pseudomonas aeruginosa* for 13.7mm and minimum of *Streptococcus sp*. 8.9mm other organism have a significance of zone of inhibition occur from the selected strain[ Figure1]. The selected strain has amplified and sequenced 1532 bp of 16S rRNA and searched by NCBI nucleotide Blast to confirmed as *Streptomyces griseus DR38,* sequence has aligned and deposited to NCBI GenBank Accession No:PP329837. The phylogenetic tree was constructed using MEGA 11.0 [8] software using various streptomyces species. The evolutionary history was inferred using the Neighbor joining method [9]. The optimal tree is shown figure 2. The evolutionary distance was computed using Maximum composite Likelihood method [10] and are in the units of the number of base substitutions per site. This analysis involved 13 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 1460bp position in the final dataset. The ethyl acetate extract was analysed by GCMS and found the Bioactive compounds are listed in table1. The presence of D-xylose is a sugar widely used as a diabetic sweetener in food and beverage process. Rutinose disaccharide sugar is attached at the 3-hydroxyl position of quercetin an interesting biological active compound, rutin is formed due to their numerous pharmacological activities ranging from antioxidant action to chronic diseases such as cancer, diabetes and inflammation [11],[12],[13] Diethyl Phthalate used for personal care products, plasticizers, cosmetics etc [14]. Secondary metabolites produced from marine actinomycets have distinct chemical structures, which may form the basis for the synthesis of new drugs. *Streptomyces griseus DR38* alone produces a wide range of metabolites having different biological activities. Enrichment and selective isolation methods can also be used to isolate rare actinomycetes from marine ecological niches having the potential to biosynthesize novel bioactive compounds. Continuous findings of novel organisms which may lead to the novel bioactive compounds.

## CONCLUSION

In this study, *Streptomyces griseus* was isolated from marine sediment soil and analyzed for its bioactive compounds using GCMS including D-xylose, rutinose and diethyl phathalate highlighting their potential pharmaceutical and nutritional application. The strain exhibited significant antibacterial activity particularly against Pseudomonas aeruginosa and streptococcus species. The 16S rRNA sequencing confirmed the identification of the strain as Streptomyces griseus DR38. The study underscores the importance of marine actinomycetes in producing novel bioactive compounds and emphasizes the need for continuous exploration of these microorganisms to discover new therapeutics agents.

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**Table 1: Identification of Bioactive compounds from *Streptomyces griseus DR38* through GCMS Analysis.**

| S.No | Compound Name | Rev. Score | Prob. % | molecular formula | molecular weight |
|---|---|---|---|---|---|
| 1 | D-Xylose | **842** | 36.92 | $C_5H_{10}O_5$ | 150.13 |
| 2 | Rutinose | 838 | 31.19 | $C_{27}H_{30}O_{15}$ | 594.5 |
| 3 | Diethyl Phthalate | 864 | 36.22 | $C_{12}H_{14}O_4$ | 222.24 |
| 4 | Phthalic acid, ethyl 2- pentyl ester | 825 | 5.71 | $C_{15}H_{20}O_4$ | 264.32 |
| 5 | Monoethyl phthalate | 868 | 5.71 | $C_{10}H_{10}O_4$ | 194.18 |
| 6 | 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester | 664 | 7.22 | $C_{24}H_{38}O_4$ | 390.6 |
| 7 | Phthalic acid, 2- methoxyethyl hexadecyl ester | 671 | 6.66 | $C_{27}H_{44}O_5$ | 448.6 |
| 8 | Phthalic acid, 2- methoxyethyl tetradecyl ester | 670 | 6.4 | $C_{25}H_{40}O_5$ | 420.6 |

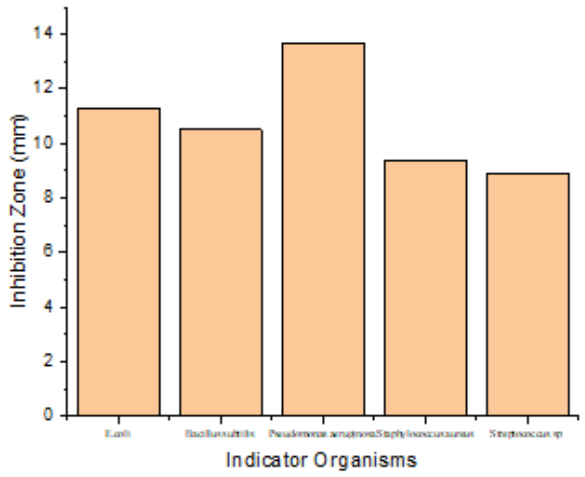


**Figure 1: Inhibition activity of *Streptomyces griseus DR38* extract against selected organisms.**

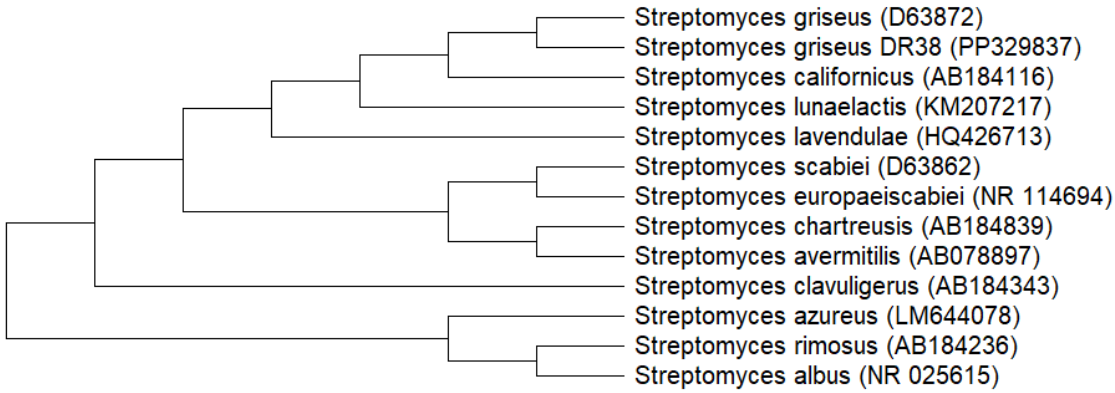


**Figure 2: Phylogenetic study of *Streptomyces griseus DR38* with Streptomyces species.**

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**RESEARCH ARTICLE**

# Antidiabetic and Antihyperlipidemic Efficacy of Veratric Acid in Streptozotocin - Nicotinamide Induced Wistar Rats

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**ABSTRACT**

Veratric acid has been shown to have multiple pharmacological activities under *in vivo* and *in vitro* conditions. The present study has made an effort to explore its antidiabetic effect in streptozotocin-nicotinamide-induced diabetic rats by analyzing the spectrum of biochemical markers as an endpoint. Diabetic animals showed a marked increase in glucose and insulin levels accompanied by drastic changes in the pattern of serum lipid profile. Veratric acid administration to diabetic animals reverted the status of glucose, insulin, and lipid profile in diabetic rats and the antidiabetic efficacy was found to be nearer to the efficacy of glibenclamide, the reference drug. The present study thus reveals the antidiabetic as well as antihyperlipidemic potential of veratric acid in diabetic rats.

**Keywords:** veratric acid, diabetes mellitus, streptozotocin, dyslipidemia.

## INTRODUCTION

Diabetes mellitus is an insulin resistance syndrome that poses significant healthcare challenges. The disease is characterized by persistent high blood sugar levels and abnormal metabolism of carbohydrates, fats, and proteins. This metabolic disruption results from inadequate secretion and/or action of insulin. High blood sugar can damage various parts of the body, including the eyes, nerves, kidneys, and heart [1,2]. An estimated 240 million people worldwide have undiagnosed diabetes, and nearly half of all adults with the ailment are unaware of it. In 2021, the

International Diabetes Federation (IDF) estimated that 537 million people had diabetes, accounting for 10.5% of the world population. Concerning, diabetes mellitus prevalence is expected to climb to 643 million (11.3%) by 2030 and 783 million (12.2%) by 2045. India has the second-highest number of diabetes patients worldwide. In the 20-79 age range, India had 74.9 million diabetics in 2021, it is possible to rise to 124.9 million by 2045 [3, 4]. Streptozotocin [2-deoxy-2-(3-methyl-3-nitrosurea) 1-D-glucopyranose] is an antibiotic derived from Streptomyces achromogenes. Its strong affinity for β-cell membranes results in selectively harmful effects on β-cells. Streptozotocin can affect pancreatic β-cells, kidneys, and liver as well as the intestine [5]. Chronic hyperglycemia is a serious condition characterized by prolonged elevated blood sugar levels [6]. While it is a hallmark of diabetes, it can also result from other medical conditions or lifestyle factors. The aetiology of type 2 diabetes mellitus involves a complex interplay between nutrition and lifestyle [7].

Dysregulation of lipid patterns is a major consequence of diabetes mellitus. Diabetes, particularly type 2, is often accompanied by characteristic changes in lipid profiles, known as diabetic dyslipidemia. This includes well-documented alterations such as increased levels of triglycerides, cholesterol, and LDL cholesterol, along with decreased levels of HDL cholesterol [8,9]. These lipid profile alterations increase the risk of cardiovascular disease in individuals with diabetes. The relationship between lipids and diabetes mellitus is a complex phenomenon, as dysregulated lipid metabolism contributes to the development and complications of diabetes [10]. Veratric acid is a phenolic phytocompound found in the stem of *Tabebuia impetiginosa* and *Sparassis crispa*, a medicinal mushroom. Fruits and vegetables contain smaller concentrations of veratric acid. This compound exhibits a variety of pharmacological effects, including anti-inflammatory, antioxidant, antimicrobial, cardioprotective, antihypertensive, and anti-hyperlipidemic activities [11]. Yu *et al*. [12] reported that veratric acid may reduce hepatic ischemia in mice. They concluded that veratric acid activates the Nrf2 signaling pathway, thereby protecting the liver from oxidative stress. Saravanakumar and Raja [13] found that veratric acid possesses notable antioxidant and anti-hyperlipidemic effects in hypertensive rats. Raja *et al*. [14] observed anti-hyperlipidemic and antioxidant activity in Wistar rats fed an atherogenic diet. Lee *et al*. [15] reported that veratric acid can prevent premature skin ageing induced by UV exposure. Choi *et al*. [16] discovered that veratric acid reduces iNOS production in macrophage cells stimulated by LPS, indicating that this inhibitory effect is mediated through histone acetylation and PI3K activation. According to Choi *et al*. [17], veratric acid regulates nitric oxide production in macrophage cells stimulated by LPS. Ran *et al*. [18] demonstrated that veratric acid effectively prevents acute lung injury in mice induced by lipopolysaccharide. This protective effect is attributed to its inhibitory influence on NF-κB expression, which mitigates inflammatory injury. Palko-Labuz *et al*. [19] discovered that conjugating veratric acid with phospholipid significantly enhanced its anticancer activity in melanoma cells. *In vitro* studies utilizing specialized colorimetric investigations revealed the free radical scavenging ability of veratric acid in KB cells [11].The present study investigates the antidiabetic and antihyperlipidemic potential of veratric acid in STZ-induced diabetic rats.

## MATERIALS AND METHODS

### Chemicals

Veratric Acid, Streptozotocin, and Nicotinamide (NA) were obtained from Sigma Aldrich Chemical Company (St. Louis, MO, USA). The rest of the chemicals were obtained from HiMediaMumbai and were of analytical grade.

### Experimental Animal model

Male albino Wistar rats weighing between 180 and 200g were maintained in the Central Animal House at Annamalai University. They have unlimited access to water and a pellet diet. The animals were kept under regulated temperatures (24 ± 2°C), humidity (50 ± 10%), and a 12-hour light/dark cycle. The experimental methodology was carried out in compliance with the Institutional Animal Ethics Committee's rules (IAEC Proposal No. Annamalai University-IAEC-1353/2/23).

### Induction of Diabetic Mellitus

Streptozotocin was dissolved in citrate-sodium buffer (0.1 M, pH 4.5), and nicotinamide was dissolved in normal physiological saline. Diabetic mellitus was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of streptozotocin (45 mg/kg b.w.), 15 minutes after the i.p. administration of nicotinamide (110 mg/kg b.w.)[20].

Treatment with veratric acid will be started on the third day in diabetic rats with a blood glucose level of more than 250 mg/dl.

**Experimental Design**

The rats were divided into five groups, each consisting of six rats. Veratric acid and glibenclamide were dissolved in 0.5% DMSO and were administrated orally to rats (1ml/rat/day) by using oral rat gavage for 30 days.

Group 1 rats served as control animals, whereas Group II rats were given streptozotocin-nicotinamide and severed as diabetic control. Group III and Group IV rats were treated with streptozotocin–nicotinamide followed by oral administration of veratric acid and glibenclamide, respectively, for 30 days. Group V rats received veratric acid treatment alone for a period of 30 days.

**Group 1:**Control rats

**Group 2:** Experimental animals were treated with a single intraperitoneal (i.p.) injection of streptozotocin (45mg/kg b.w.), 15 minutes after the i.p. injection of nicotinamide (110mg/kg b.w.) (Diabetic control rats)

**Group 3:**Diabetic rats were treated with veratric acid (40mg/kg b.w.).

**Group 4:**Diabetic rats were treated with glibenclamide (600µg/kg body weight).

**Group 5:** Experimental animals were treatedwith veratric acid alone (40mg/kg body weight).

**Biochemical Analysis**

The rats were killed at the end of the experiment, and blood samples were taken. Centrifugation yields plasma or serum. The pancreas, kidney, and liver were then removed, homogenized with suitable buffers, and centrifuged. The resultant supernatants were used to estimate biochemical parameters. Additionally, a part of the tissues was set aside for histological examination.

**Estimation of blood glucose, plasma insulin, hemoglobin, and glycosylated hemoglobin:**

Blood glucose levels were measured using glucose oxidase-peroxidase techniques [21]. Blood samples were taken from the tail vein, and glucose levels were evaluated with a glucometer. Hemoglobin levels in the blood were estimated using the Drabkin and Austin method (1932) [22], while HbA1c (glycosylated hemoglobin) levels were measured using the Bannon method (1982) [23]. Plasma insulin levels were determined using an enzyme-linked immunosorbent assay (ELISA) with a Boehringer Mannheim kit and a Boehringer analyzer ES300 [24].

**Evaluation of Serum Lipids**

Triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels were measured in serum using kits from Swemed Biomedicals Pvt Ltd., Bengaluru, India. Friedewald's algorithm [25,26] was used to calculate LDL and VLDL cholesterol levels.

LDL cholesterol = (Total Cholesterol - HDL cholesterol) - (Triglycerides / 5)

VLDL cholesterol = Triglycerides / 5

**Histopathology Studies**

The liver, kidneys, and pancreas were first fixed in 10% neutral buffered formalin. They were then dehydrated by passing through a gradient of ethyl alcohol-water combinations. The samples were dehydrated, washed with xylene, and then embedded in paraffin. Organ sections (5µm thick) were stained with hematoxylin and eosin dye and examined under a light microscope [27].

**Statistical Analysis**

The data is shown as mean ± SD. The statistical studies employed one-way analysis of variance (ANOVA), with post-hoc comparison using Duncan's Multiple Range Test (DMRT). A p-value of less than 0.05 indicated statistical significance.

## RESULTS

**Estimation of blood glucose, plasma insulin, hemoglobin, and glycosylated hemoglobin levels**

Table 1 shows the concentrations of blood glucose, plasma insulin, haemoglobin, and glycosylated haemoglobin. The study found significant variations in the levels of blood glucose, plasma insulin, haemoglobin, and glycosylated haemoglobin between diabetic animals and those treated with veratric acid. In comparison to control rats, diabetic animals had higher levels of blood glucose and glycosylated haemoglobin, as well as lower levels of insulin and haemoglobin. However, oral treatment of veratric acid at a dose of 40mg/kg body weight to diabetic rats recovered these metabolic characteristics. The efficacy of veratric acid was comparable to that of the standard drug glibenclamide

**Evaluation of Serum Lipids**

Figure 1 illustrates the serum lipid pattern observed in experimental animals. In diabetic animals, significant increases were observed in total cholesterol, LDL cholesterol, and triglycerides, while the level of HDL cholesterol was notably decreased compared to control rats. Veratric acid administration to diabetic rats dramatically recovered total cholesterol, triglyceride, HDL, LDL, and VLDL levels to a near-normal range. Furthermore, the antihyperlipidemic effect of veratric acid was found to be comparable to that of glibenclamide.

**Histopathology Studies**

Figures 2, 3 and 4 depict the histopathological changes observed in the liver, pancreas, and kidney of experimental animals. Marked histological alterations were observed in the liver of diabetic animals compared to control rats. However, oral administration of veratric acid to diabetic rats reversed these histopathological abnormalities, with its effect being comparable to that of glibenclamide. Control rats and rats that received veratric acid alone exhibited a normal architecture of the liver, pancreas, and kidney.

## DISCUSSION

Prominent researchers have utilized STZ-NA-induced diabetes mellitus to investigate the antidiabetic efficacy of medicinal plants and their bioactive principles [28]. Nicotinamide plays a crucial role in reducing the severity of pancreatic β-cell damage during STZ-NA-induced diabetes mellitus, thus mimicking the model of type II diabetes mellitus [29]. STZ-NA-induced diabetes is histologically similar to type II diabetes mellitus. In the current investigation, diabetic rats exhibited hyperglycemia, defined as blood glucose levels greater than 250 mg/dL. Veratric acid given to diabetic rats lowered not only blood glucose levels, but also the degree of histological abnormalities in the liver, pancreas, and kidney. Veratric acid administered to diabetic rats dramatically lowered blood glucose levels, demonstrating its antihyperglycemic efficacy against STZ-NA-induced diabetes mellitus. Glycosylated hemoglobin has been considered a good diagnostic marker in diabetes mellitus[30]. The present study observed an increase in glycosylated hemoglobin in diabetic rats and a decrease in diabetic rats treated with veratric acid, which further validates its antidiabetic effect.

Diabetic dyslipidemia is recognized as a major complication of diabetes mellitus, with a significant association with cardiovascular diseases. Elevated triglyceride levels, along with increased LDL cholesterol and decreased HDL cholesterol, are primary factors contributing to diabetic dyslipidemia. Insulin deficiency alters the status of various lipid-metabolizing enzymes, thereby contributing to lipid abnormalities in diabetes mellitus [31,32]. Extensive studies have documented lipid abnormalities in both human subjects and experimental animal models, including streptozotocin-induced diabetic rats [33,34]. In this study, STZ-NA-induced diabetic rats demonstrated notable abnormalities in their serum lipid profile compared to control animals. Elevated levels of cholesterol, LDL cholesterol, and triglycerides, alongside decreased concentrations of HDL cholesterol, pointed towards dyslipidemia in diabetic rats. Insulin insufficiency and hyperglycemia in diabetic rats have been suggested to have contributed to the development of diabetic dyslipidemia. Administration of veratric acid to diabetic rats significantly decreased serum levels of total cholesterol, triglycerides, and LDL cholesterol. Furthermore, veratric acid stimulated insulin secretion and increased the concentration of HDL cholesterol in diabetic rats. These findings from the present

investigation highlight the antihyperlipidemic potential of veratric acid in STZ-induced diabetic rats. The antidiabetic efficacy of veratric acid was compared with the standard reference drug glibenclamide. The study observed that the antidiabetic potential of veratric acid was comparable to that of glibenclamide. Veratric acid significantly restored blood glucose levels, and serum lipid patterns, and reduced the severity of histopathological abnormalities in STZ-NA-induced diabetes mellitus in Wistar rats, like glibenclamide. These findings revealed the significant antihyperglycemic and antihyperlipidemic potential of veratric acid in STZ-NA-induced diabetic rats.

## CONCLUSION

Veratric acid reduces blood glucose levels, glycosylated haemoglobin, total cholesterol, and triglycerides in STZ-NA-induced diabetic rats. Veratric acid's antidiabetic and antihyperlipidemic advantages may be due to its ability to promote insulin release from diabetic rats' pancreatic β-cells.

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**Table 1. The concentration of blood glucose, plasma insulin, hemoglobin and glycosylated hemoglobin Each value represents the mean ± SD for six rats in each group. Statistical significance, denoted by different superscript letters (a–e), indicates differences at p < 0.05 (DMRT).**

| Parameters | Control | Diabetic Control | Diabetic Rats + Veratric acid (40mg/kg b.w) | Diabetic Rats + Glibenclamide (600µg/kg b.w) | Veratric acid alone (40mg/kg b.w) |
|---|---|---|---|---|---|
| Blood glucose (mg/dl) | 82.16 ± 4.62 [a] | 264 ± 9.34 [b] | 102.50 ± 4.08 [c] | 112 ± 5.04 [c] | 88 ± 4.57 [a] |
| Plasma Insulin (µU/ml) | 18.64 ± 2.26 [a] | 7.02 ± 2.78 [b] | 15.21 ± 1.89 [c] | 16.07 ± 2.51 [c] | 17.07 ± 2.58 [a] |
| Hemoglobin (g/dl) | 14.95 ± 1.56 [a] | 7.63 ± 1.67 [b] | 12.75 ± 1.72 [c] | 13.05 ± 1.21 [c] | 14.16 ± 1.53 [a] |
| HbA1c (%) | 5.82 ± 0.35 [a] | 11.06 ± 0.53 [b] | 7.16 ± 0.26[c] | 8.56 ± 0.28 [c] | 6.08 ± 0.46 [a] |

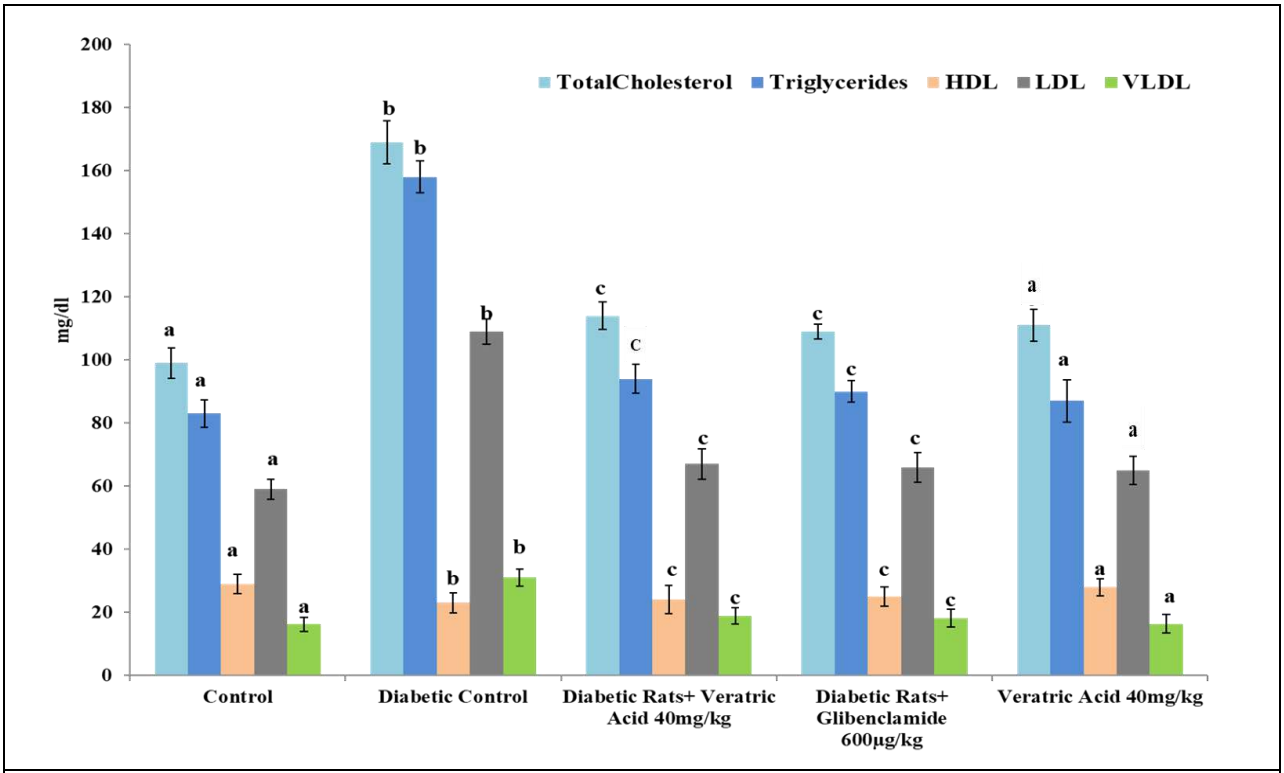


**Figure 1.Effect of veratric acid in serum lipid profile in experimental rats**
**Each value represents the mean ± SD for six rats in each group. Statistical significance, denoted by different superscript letters (a–e), indicates differences at p < 0.05 (DMRT).**

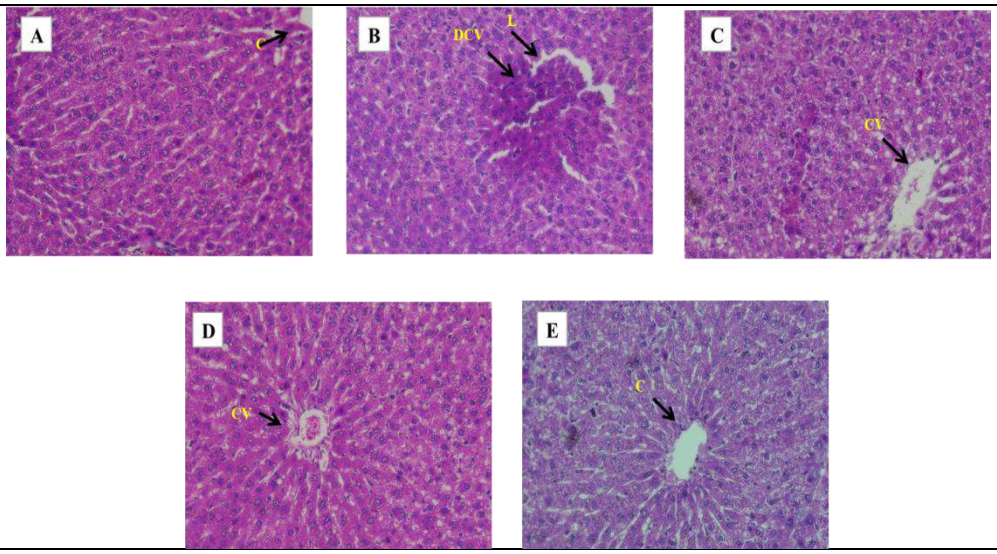


**Figure 2. Histological observation of liver in normal and experimental rats. C-Central Vein; CV-Congestive Vessel; DCV- Dilated Central Vein; L- Lymphocytic Infiltration. A: Control rats showed the normal histological architecture of liver tissue (Group 1). B: Diabetic control rats showed lymphocytic infiltration (L) and inflammation with a dilated central vein (DCV) (Group 2). C: Diabetic rats treated with veratric acid (40 mg/kg b.w.) showed congested hepatic vessels (CV) and mild vascular changes in hepatocytes (Group 3).**
**D: Diabetic rats treated with glibenclamide (600 µg/kg b.w.) showed normal architecture of hepatic vessels (Group 4). E: Control rats treated with veratric acid alone (40 mg/kg b.w.) showed similar findings to the control group (Group 5). Examinations were carried out at 40X magnification with hematoxylin-eosin staining.**

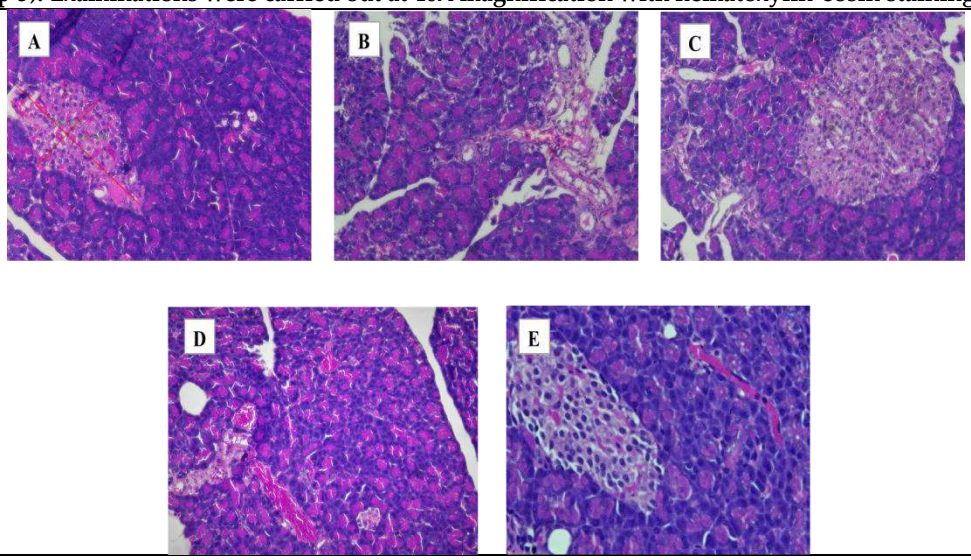


**Figure 3.Histopathological observation of pancreas in normal and experimental rats. A: Control group showed normal cellular architecture (Group 1). B: Diabetic control rats showed a significant reduction in islet volume, with clear signs of necrosis observed in the islets of Langerhans (Group 2). C: Diabetic rats treated with veratric acid (40 mg/kg b.w.) showed a normal pancreatic morphology with reduced numbers of pancreatic islets cells. The changes in nuclear morphology with hyperchromatic and mild dysplasia was noticed(Group 3). D: Diabetic rats treated with glibenclamide (600 µg/kg b.w.) showed normal pancreatic morphology with slight alteration in islets cells (Group 4). E: Control rats treated with veratric acid alone (40 mg/kg b.w.) showed normal β cells with normal cellular architecture. Examinations were carried out at 40X magnification with hematoxylin-eosin staining.**

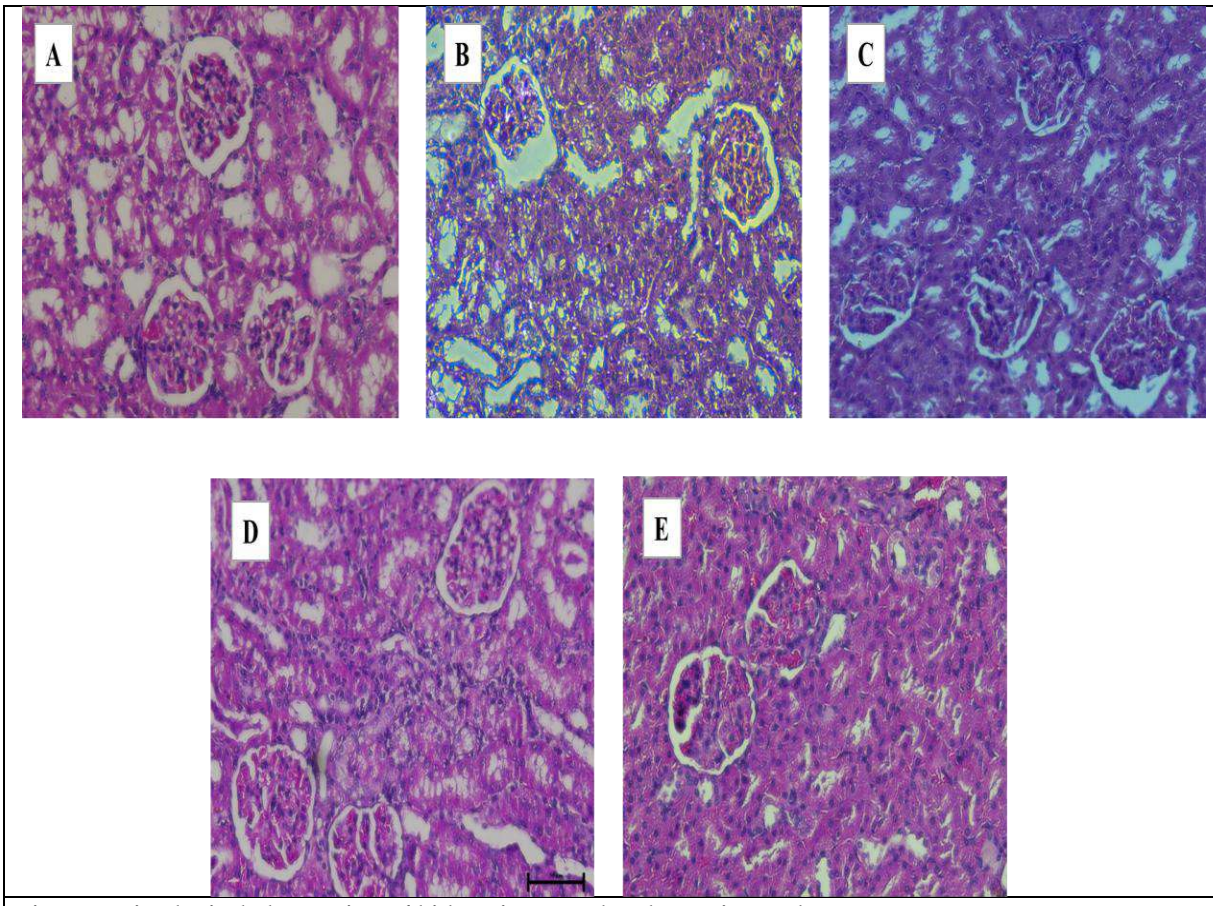


**Figure 4:Histological observation of kidney in normal and experimental rats.**
**A: Control group showed normal glomerular and tubular morphology (Group 1).**
**B: Diabetic control rats showed glomerular congestion and reduced vascular spaces. The tubular structure appeared edematous with signs of derangement (Group 2).**
**C: Diabetic rats treated with veratric acid (40 mg/kg b.w.) exhibited normal glomerular appearance, increased vascularity, and capsular space. Tubular regions showed mild congestion (Group 3).**
**D: Diabetic rats treated with glibenclamide (600 µg/kg b.w.) demonstrated glomerular hypertrophy with increased vascularity. The tubules exhibited signs of congestion and derangement (Group 4).**
**E: Control rats treated with veratric acid alone (40 mg/kg b.w.) showed increased glomerular vascularity and normal tubular morphology (Group 5). Examinations were carried out at 40X magnification with hematoxylin-eosin staining**

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**RESEARCH ARTICLE**

# Antioxidant and Antiglycation Properties of *Syzygium aromaticum* bud: Potential Therapeutic Applications in Diabetes Management

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## ABSTRACT

This study delves into the antioxidant, antidiabetic, and antiglycation properties of the aqueous extract of Syzygium aromaticum (clove) bud. The extract demonstrates significant bioactive potential with its high total phenolic content (30.50 ± 0.05 mg GAE/g DW) and flavonoid content (19.80 ± 0.04 mg QE/g DW). These compounds contribute to its robust antioxidant capacity, as evidenced by the low IC50 values in DPPH (75 ± 0.08 μg/mL) and ABTS (3.9 ± 0.05 μg/mL) assays, and substantial ferric reducing antioxidant power (2.10 ± 0.40 mmol $Fe^{2+}$/g DW). The extract's nitric oxide scavenging activity further supports its potential to mitigate oxidative stress and inflammation, providing reassurance about its therapeutic potential. The clove bud extract, with its robust anti-diabetic properties, inhibits key carbohydrate-digesting enzymes, with IC50 values of 90 ± 0.07 μg/mL for $\alpha$-amylase and 120 ± 0.06 μg/mL for $\alpha$-glucosidase, indicating its efficacy in managing postprandial blood glucose levels. Additionally, the extract exhibits significant antiglycation activity, reducing browning by 38.50% ± 10.05, fructosamine production by 50.00% ± 4.50, protein carbonyl content by 48.20% ± 0.40, amyloid β-structure formation by 43.50% ± 11.00, and total AGEs formation by 83.00%. These results underscore its potential in preventing diabetic complications, reinforcing the audience's confidence in its effectiveness.

**Keywords:***Syzygium aromaticum* (clove), Advanced glycation end products (AGEs), Reactive oxygen species, Antioxidant, Oxidative stress.

# INTRODUCTION

Diabetes is a prevalent health issue affecting approximately 425 million individuals globally, with India alone accounting for around 72 million cases (International Diabetes Federation, 2017). This chronic condition, diabetes mellitus, is characterised by persistent high blood sugar levels. A key feature of both Type 1 and Type 2 diabetes is insulin resistance, which hampers glucose uptake in the body, particularly in skeletal muscles, leading to impaired glucose transport. Additionally, heightened activity of the pancreatic enzymes alpha-amylase and alpha-glucosidase further elevates blood glucose levels.(Bhatnagar & Mishra, 2022). Obesity often accompanies diabetes, with about 90% of those with insulin resistance being overweight (BMI ≥ 25 kg/m²) (Amisi, 2022). The American Medical Association classified obesity as a chronic disease in 2013. Long-term diabetes can result in oxidative stress, which is linked to complications such as retinopathy, nephropathy, neuropathy, cardiomyopathy, ageing, rheumatoid arthritis, and osteoporosis(Reddy & Tan, 2020).

*Syzygium aromaticum* (clove) is highly regarded in culinary and medicinal fields due to its rich phytochemical composition. Clove contains essential oils, including eugenol, with significant antioxidant and anti-inflammatory properties. (Haro-González et al., 2021). Additionally, clove is known for its antimicrobial, antifungal, and antiviral activities. The buds of *Syzygium aromaticum* are used extensively for their therapeutic benefits, including their ability to manage blood sugar levels and prevent oxidative stress(Nait Irahal et al., 2023). Clove's safety and efficacy have been supported by its long history of use in traditional medicine across various cultures(Idowu et al., 2021). Given its diverse bioactive components, *Syzygium aromaticum* has been studied for its potential benefits in mitigating oxidative stress and managing diabetes-related complications. This study aims to analyze the phytochemical content, antioxidant capacity, and antiglycation properties of *Syzygium aromaticum* (clove) and examine their interrelationships to provide a comprehensive understanding of its therapeutic potential.

# METHODOLOGY

**Extract Preparation**

The aqueous extract was prepared using the protocol described earlier(Abubakar & Haque, 2020), with some modifications. The powdered clove buds (5 g) of uniform particle size were added to distilled water (50 mL), vortexed for 1 h in a water bath at 100 °C, and centrifuged for 15 min at 4500 rpm. The bud powder (5 g) of uniform particle size was mixed in 50 mL of methanol or ethanol and kept in a shaking incubator at 200 rpm for 24 h. The solutions obtained were filtered with Whatman No. 1 filter paper and stored at 4–8 °C until further use.

**Determination of Total Phenolic (TPC) and Flavonoid Content (TFC)**

The phenolic and flavonoid contents were obtained for all extracts by using the Folin–Ciocalteu method and the aluminium chloride method(Phuyal et al., 2020), respectively. A 250 μL volume of the extract (10 mg/mL) was used for all assays. For TPC measurement, the standard curve of gallic acid (0–250 μg/mL) was used and the results were demonstrated in mg of gallic acid equivalents (GAE)/g DW of a sample ($y = 0.008x + 0.026$; $R^2 = 0.991$). Similarly, for TFC measurement, a standard curve of quercetin (0–100 μg/mL) was plotted and the results were mentioned in mg of quercetin equivalents (QE)/g DW of the sample ($y = 0.026x + 0.038$; $R^2 = 0.998$)

**Reactive Oxygen and Nitrogen Assays**

**Antioxidative Assays**

The antioxidative properties of the extracts were evaluated using several methods. The DPPH assay followed the protocol by Chaves et al. (2020), the ABTS assay was based on Xiao et al. (2020), and the FRAP assay was conducted as described by Prastiwi et al. (2020).

**Nitric Oxide Scavenging Assay**

The nitric oxide scavenging activity was measured using the method described by Ali et al. (2020). Various concentrations of extract (0–500 μg/mL) were incubated at 25 °C for 2.5 hours.

**Antidiabetic Assays**

The antidiabetic potential of the extracts was evaluated using $\alpha$-amylase and $\alpha$-glucosidase inhibitory assays. The $\alpha$-amylase inhibitory activity was assessed according to Khadayat et al. (2020), involving the pre-incubation of 100 μL of extract and 250 μL $\alpha$-amylase solution (100 U/mL) at 37 °C for 10 minutes, followed by the addition of 250 μL of 10% starch. The mixture was incubated at 37 °C for 10 minutes, DNS was added, and absorbance was measured at 595 nm. The $\alpha$-glucosidase inhibitory activity followed the protocol by Kifle et al. (2021), where 100 μL of $\alpha$-glucosidase (100 U/mL) was preincubated with 50 μL of the extract for 10 minutes. The reaction was initiated with 5 mM pNPG and stopped with sodium carbonate, with absorbance measured at 405 nm.

**Antiglycation Assays**

The antiglycation activity of the extracts was evaluated using several methods. The BSA assay was used to determine antiglycation activity with modifications from Muraoka et al. (2022). Browning intensity was measured at 420 nm as per Fallavena et al. (2022). Fructosamine content was evaluated using the NBT assay based on Mohd Dom et al. (2020). Protein carbonyl content was determined using the DNPH methodology described by Nareshkumar et al. (2020). Amyloid β-structure estimation followed the Congo red binding method from Gao et al. (2021). Total AGEs were estimated using a BSA-fluorescent assay as per Dedvisitsakul & Watla-Iad (2022).

## RESULTS AND DISCUSSION

**Determination of TPC and TFC**

The total phenolic (TPC) and total flavonoid (TFC) of *Syzygium aromaticum* extract were quantified. The extract exhibited a TPC of 30.50 ± 0.05 mg GAE/g DW and a TFC of 19.80 ± 0.04 mg QE/g DW, indicating high concentrations of these compounds known for their antioxidant properties (Muflihah et al., 2021; Hassan et al., 2021; Dev et al., 2021).

**Reactive Oxygen and Nitrogen Assays**

The DPPH assay showed an IC50 value of 75 ± 0.08 μg/mL, indicating strong antioxidant capacity, superior to *Rosmarinus officinalis* (Kamli et al., 2022). The ABTS assay yielded an IC50 value of 3.9 ± 0.05 μg/mL, more effective than *Ocimumbasilicum* (Nguyen et al., 2021). The FRAP assay showed a value of 2.10 ± 0.40 mmol $Fe^{2+}$/g DW, slightly lower than *Punica granatum* (Sabraoui et al., 2020). The nitric oxide scavenging assay demonstrated an IC50 value of 350 ± 0.10 μg/mL, better than *Curcuma longa* (Lucas et al., 2021; Cheikhyoussef et al., 2022).

**Antidiabetic Assays**

The extract inhibited $\alpha$-amylase with an IC50 value of 90 ± 0.07 μg/mL, more potent than *Cinnamomum zeylanicum* (Niroshani et al., 2020). The inhibition of $\alpha$-glucosidase had an IC50 value of 120 ± 0.06 μg/mL, better than Ficus carica (Meziant et al., 2021).

**Antiglycation Assays**

The extract reduced browning by 38.50% ± 10.05, indicating strong antiglycation activity, similar to *Nigella sativa* (Balyan et al., 2022). It reduced fructosamine production by 50.00% ± 4.50, outperforming *Pterocarpus marsupium* (Dinda, 2022). The reduction in protein carbonyl content was 48.20% ± 0.40, more effective than Allium sativum (Ncir et al., 2020). The extract reduced the protein aggregation index to 43.50% ± 11.00, comparable to *Bacopa monnieri* (Awathale et al., 2021). It reduced total AGEs formation by 83.00%, surpassing Trigonella foenum-graecum (Hafeez et al., 2023).

## DISCUSSION

The findings of this study highlight the significant therapeutic potential of *Syzygium aromaticum* (clove) bud extract in managing oxidative stress, diabetes, and glycation-associated complications. The high total phenolic (30.50 ± 0.05 mg GAE/g DW) and flavonoid (19.80 ± 0.04 mg QE/g DW) content of the clove bud extract underscores its robust antioxidant capacity, as evidenced by low IC50 values in both DPPH (75 ± 0.08 μg/mL) and ABTS (3.9 ± 0.05 μg/mL) assays. These values surpass those of commonly studied plant extracts like *Rosmarinus officinalis* and *Ocimum basilicum*, indicating a superior radical scavenging ability (Muflihah et al., 2021; Nguyen et al., 2021; Kamli et al., 2022). The FRAP assay results further confirm the substantial electron-donating ability of the extract, contributing to its overall antioxidant efficacy (Sabraoui et al., 2020).

The extract's nitric oxide scavenging activity (IC50 = 350 ± 0.10 μg/mL) demonstrates its potential to mitigate nitrosative stress and inflammation, which is crucial in managing chronic inflammatory diseases. Compared to extracts like *Curcuma longa*, the clove bud extract shows a more potent nitric oxide scavenging activity, highlighting its effectiveness in reducing oxidative stress (Lucas et al., 2021; Cheikhyoussef et al., 2022). In the context of diabetes management, the clove bud extract exhibits strong inhibitory effects on key carbohydrate-digesting enzymes, $\alpha$-amylase (IC50 = 90 ± 0.07 μg/mL) and $\alpha$-glucosidase (IC50 = 120 ± 0.06 μg/mL). These findings suggest that the extract can effectively slow down carbohydrate digestion and absorption, thereby managing postprandial blood glucose levels. The dual inhibition of these enzymes is particularly noteworthy as it highlights the comprehensive antidiabetic potential of the extract, comparable or even superior to other known natural inhibitors like *Cinnamomum zeylanicum* and *Ficus carica* (Niroshani et al., 2020; Meziant et al., 2021).

The antiglycation assays further establish the efficacy of the clove bud extract in preventing diabetic complications. The significant reduction in browning, fructosamine production, protein carbonyl content, amyloid β-structure formation, and total AGEs formation underscores the extract's potential in inhibiting glycation processes (Balyan et al., 2022; Dinda, 2022; Ncir et al., 2020; Awathale et al., 2021; Hafeez et al., 2023). These properties are critical for managing the progression of diabetic complications and other ageing-related disorders associated with AGEs. Overall, the multifunctional bioactive properties of *Syzygium aromaticum* bud extract make it a promising natural therapeutic agent. Its potent antioxidant, antidiabetic, and antiglycation activities provide a compelling case for its inclusion in developing interventions to manage oxidative stress, diabetes, and related complications. Further studies exploring its molecular mechanisms and clinical efficacy could pave the way for its application in functional foods and pharmaceuticals.

## CONCLUSION

The aqueous extract of *Syzygium aromaticum* bud emerges as a potent natural agent with multifaceted health benefits, including antioxidant, antidiabetic, and antiglycation. These findings support its potential application in developing therapeutic interventions for oxidative stress-related conditions, diabetes management, and the prevention of glycation-associated complications. Further studies and clinical evaluations are warranted to fully elucidate its mechanisms and optimise its use in health promotion and disease prevention.

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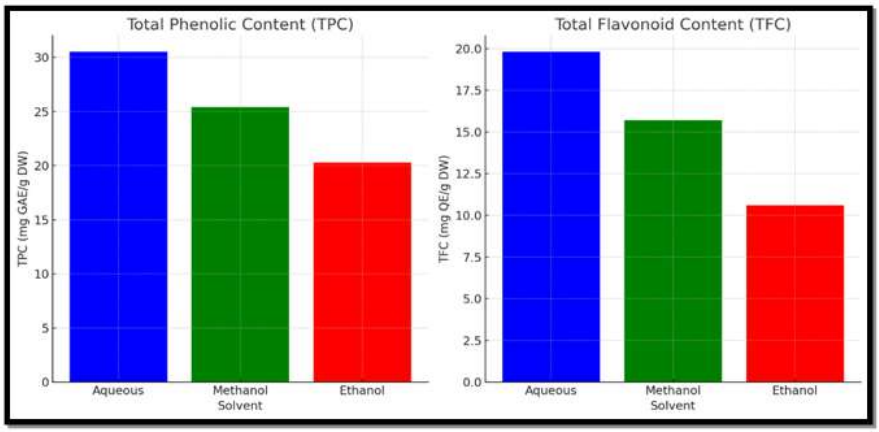


**Figure: 1 Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Various Extracts of *S.aromaticum Bud***

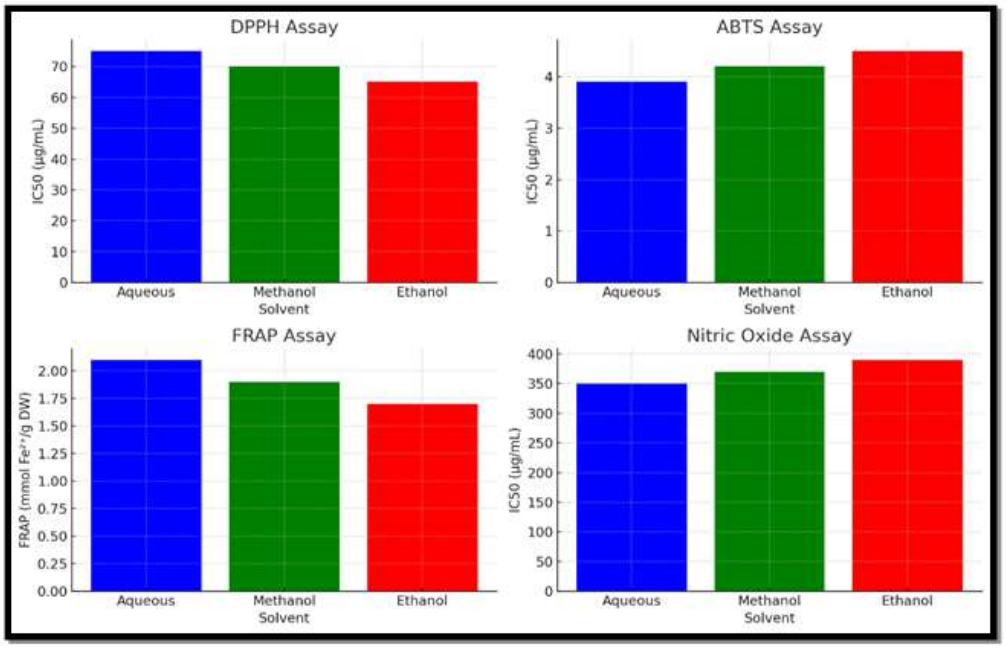


**Figure 2: Different Antioxidant Confirming Assays for Extracts of *S.aromaticum* Bud**

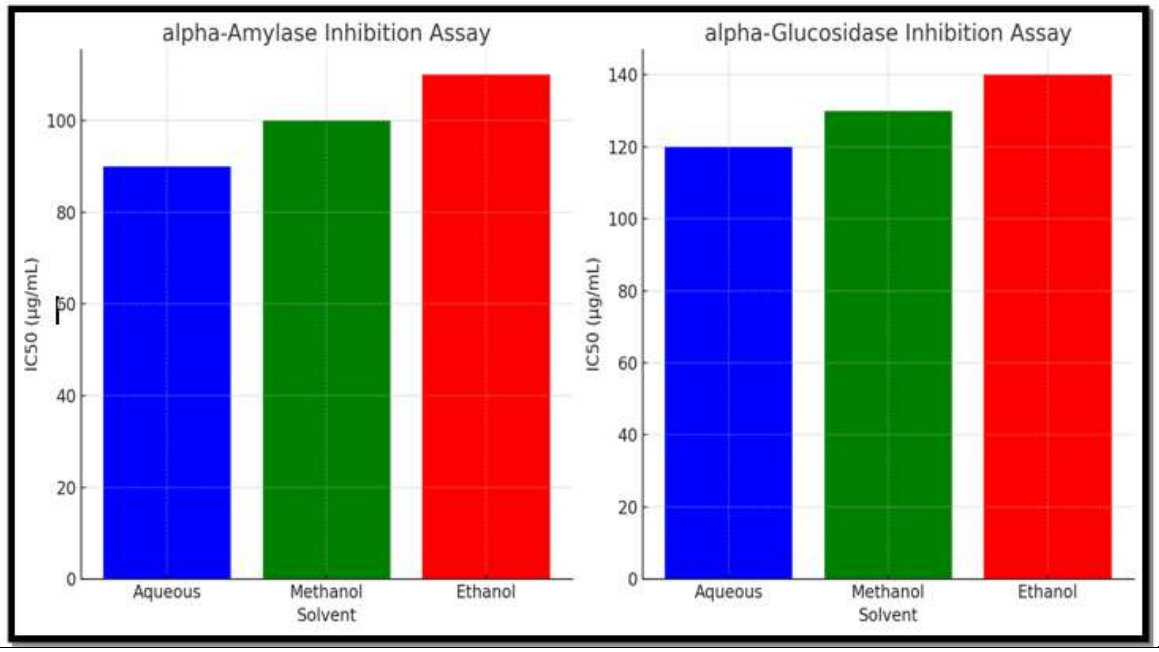


**Figure 3: In Vitro Anti-Diabetic Activities of Extracts of *S.aromaticum* bud**

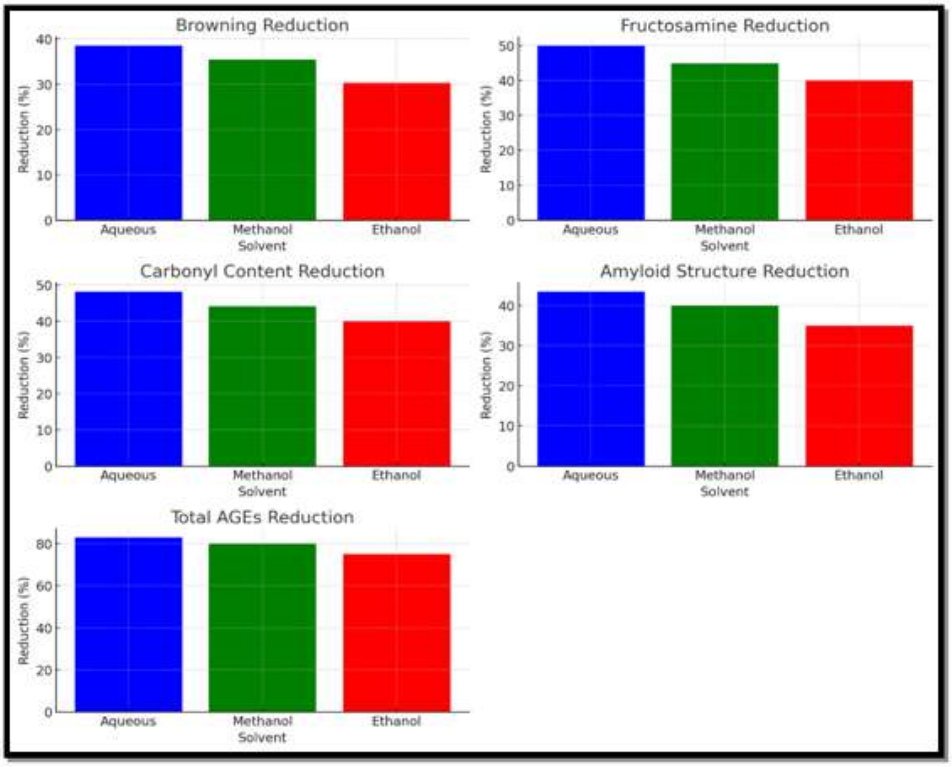


**Figure: 4 Anti-Glycation Properties of Extracts of *S.aromaticum* Bud**

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**RESEARCH ARTICLE**

# GC-MS Analysis of *Canscora alata* (Roth) Wall- a Potent Medicinal Plant

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## ABSTRACT

In the present study, the bioactive phytoconstituents of *Canscora alata* (Roth) Wall whole plant extracts has been analysed using GC-MS technique. The chemical constituents of petroleum ether, hexane, ethylacetate and ethanol extracts were identified using the database of National Institute of Standards and Technology (NIST). The database which is universal and consists of over 62,000 different patterns of already identified and known bioactive compounds. The major peak area possessing constituents identified in petroleum ether extract are Tetracosane 24.179, Hexadecanoic acid, butyl ester 24.031, Z and Z-8,10-Hexadecadien-1-ol 23.737 and Tetrapentacontane 23.425, the hexane extract identified in major peak phytoconstituents are Z, Z-6,28-Heptatriactontadien-2-one having maximum peak percentage area of 24.765, Tetrapentacontane peak area of 24.188, Octadecanoic acid contain 23.949, Tetracosane 24.179 and Undec-10-ynoic acid, tetradecyl ester having maximum peak percentage area of 24.789 (0.50%), The major peak area possessing constituents identified in ethylacetate are E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate having maximum peak percentage area of 24.766, 4H-Pyrazole-4-carbonitrile, 3-[[(2-hydroxy-3-methoxyphenyl) methyl] amino]- 24.193 and Octadecanoic acid 23.929 and the ethanol extract possessing the major peak value in 24.789 (0.50%), 1,3-Bis(2-pyrazinoxy) propane – 24.298 (0.66), Octadecanoic acid, ethyl ester – 24.119(0.50), Octadecanoic acid-23.978(2.05), 7-Hexadecyn-1-ol - 23.825(19.55) and 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-.

**Keywords**: *Canscora alata*, Gentianaceae, pharmacological activity, Hexadecanoic acid, methanol ester, antioxidant, antiinflamatory.

## INTRODUCTION

The use of medicinal plant goes back to the beginning of human life on earth and exploiting the plants of their natural surroundings. Nowadays looking to natural products for bioactive compounds and new drug discovery is considerable. Traditional medicine consists of a lot of valuable practical information in conjunction with the therapeutic principles and using materials including herbs, as therapeutic agent. The medicinal value of herbal plants lies in their bioactive principles as plant constituent that produce definite physiological action on the human body [Akinmoladun *et al*., 2007]. Some of them are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds. These metabolites are the actual determinants of their pharmacological properties [Edeoga *et al*., 2005 and Panday and Rizvi,2009]. Today we know the modern prescription drugs are from natural compounds [Goh *et al*., 1995]. Researchers have thus turned to traditional medicine, which is still used widely throughout the world. *Canscora alata* (Roth)Wall. is one of the medicinally important plant belonging to the family Gentianaceae. *Canscora decussata* is the synonym of *Canscora alata*. In India, *Canscora decussata* Schult. is popularly known as "Shankhpushpi" and found throughout India, up to an altitude of 1300 m. It is also grown in Sri Lanka and Myanmar.[Sethiya, N.K. *et al*.,2010]. It is an important Ayurvedic drug, is reputed as an alternative, laxative and brain tonic. It occurs as an important ingredient in Brahmarasayana, Aindrarasayana, Agastyaharitaki, Medhyarasayana, Manasamitram and in a number of other composite drugs. This plant grows up to 20cm, stems basally and apically branched, with prominent wings up to 1.6 mm wide. Leaves are cauline and sessile, lamina with three main veins, elliptic to lanceolate shape and often falcate. The base is wedge shaped to shortly attenuate with acute apex. Inflorescences are axillary, cymes; bracts linear to lanceolate, pedicels 2–26 mm long. Calyx persistent, with eight prominent, 1–1.3 mm wide wings; tube 8–9 mm long; lobes anisomorphic which are acute, two larger lobes are long and two lobes are shorter. Corolla tube 8–9 mm long, white, two lobes broadly elliptic and the other two lobes are elliptic. Filament of upper stamen are 1.8–2.2 mm long, broadened below anther. Ovary 6–8 style up to 4 mm long; stigmatic lobes 0.7. Capsule 8. Seeds irregular in shape Plate-1. From the literature the study plant is usually used in various ailments, such as nervine tonic and memory invigorator. It is also used in cerebral abnormalities, insomnia, burning sensation odema and Urinary disorders. Medicinal plants serve as therapeutic alternatives, safer choices or on some cases, as the only effective treatment. People in different cultures and places have used particular plants to treat certain medicinal problems. A larger number of these plants and their extract have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer and antimicrobial effects [Huffman, 2003; Miller *et al*., 2004; Thatte *et al*., 1992 and Sethiya,N.K.,*et al*., 2010].Therefore, in present investigation, we analysed the GC-MS analysis of *Canscora alata* extracts.

## MATERIALS AND METHODS

**Plant material and collection**

The healthy whole plants of *Canscora alata* (Roth) was selected and collected from Vellode, Erode District, Tamil Nadu, India, during September 2021. The identity of the plant specimen was confirmed by using the Floras [Gamble and Fischer, 1956; Matthew, 1983] and Botanical Survey of India, Southern region center, Coimbatore, Tamil Nadu. The herbarium number provided by Botanical survey of India is "BSI/SRC/5/23/2021/Tech/295".

**Preparation of powder**

The shade dried entire plant was mechanically ground into coarse powder and passed through 80 mesh sieve and used for extraction process.

**Preparation of extracts**

The plant powder was extracted successively using Soxhlet apparatus with petroleum ether (60-80 °C), hexane (63-70 °C), ethylacetate (77.1 °C) and ethanol (99.9 °C). Each time before extracting with next solvent, powdered

material were dried in an air oven below 50°C. The extracts were dried over anhydrous sodium sulfate, stored in sealed vials in refrigerator (5-8°C) until analysis.

**GC-MS Analysis** (Jayanthi and Jegadeesan,2008)

GC-MS analysis of petroleum ether,hexane, ethylacetate and ethanol extracts were performed in therma GC- Trace Ultra ver:5.0 and a Gas Chromatography interface to a Mass Spectrometer equipped with a thermo MS DS Q II fused a DB35-MS capillary standard non polar column (30x0.25µm ID x 0.25 µm df). For detection, an impact mode with ionization energy of 70 ev. Helium was used as a carrier gas at a constant flow rate of 1 ml/minutes, and an injection volume of 1µm was employed (a split ratio of 10:1). The injector temperature was maintained at 2500C, the oven temperature was programmed from 1500C for 2 minutes, with an increase of 50C/minutes to 2200C, then 100C/minutes to 2600C, ending with a 20 minutes isothermal at 3500C. Mass Spectra were taken at 70ev. The scan interval of 0.5 sec and fragments from 50 to 650 Da. The solvent delay was 0 to 4 minutes and the total GC-MS running time was 37.50 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas.

**Identification of compounds**

In order to identify the various bioactive components and interpretation of Mass Spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database which is universal and consists of over 62,000 different patterns of already identified and known bioactive compounds. The spectra of the unknown component of petroleum ether, hexane, ethylacetate and ethanol extracts of were compared with the standard existing Mass Spectra of known components already stored in NIST library.

## RESULTS

The obtained chromatogram of hexane, petroleum ether, ethylacetate, and ethanol are given in fig 1,2,3 and 4. respectively. The hexane extract contains 87 constituents, petroleum ether extract yielded 89 constituents, ethylacetate has 59 constituents and the ethanol has 57 constituents. The obtained phytoconstituents names with their retention time (RT), molecular formula, molecular weight (MW) and concentration in area (%) are presented in Table-1,2,3 and 4 respectively. The petroleum ether extract contains totally 89 components. The main active principles present in petroleum ether extract are Tetracosane 24.179, Hexadecanoic acid, butyl ester 24.031, Z, Z-8,10-Hexadecadien-1-ol 23.737 and Tetrapentacontane 23.425, and the minimum compounds presents in Petroleum ether extract are Ethylbenzene 3.669, o-Xylene 3.856, Benzene, 1-azido-2-methyl- 4.427 and Benzene, propyl- 6.136 (Table-1).

The hexane extract contains about 87 compounds in which the major components are Z, Z-6,28-Heptatriactontadien-2-one having maximum peak percentage area of 24.765, Tetrapentacontane peak area of 24.188, Octadecanoic acid contain 23.949, 11-Dodecyn-1-ol acetate 23.790 and Tetrapentacontane 23.430.The minimum compounds present in hexane extract was 1,3,5,7-Cyclooctatetraene 4.361, Benzene, 1-ethyl-2-methyl- 6.348, Mesitylene 6.576 (Table-2). The ethylacetate extract contains about 59 compounds in which E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate having maximum peak percentage area of 24.766, 4H-Pyrazole-4-carbonitrile, 3-[[(2-hydroxy-3-methoxyphenyl) methyl] amino]- 24.193, Octadecanoic acid 23.929, 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- 23.756 and Phytyl stearate 23.354 and the minium peak values are 3, Trans-(1,1-dimethylethyl)-4, cis-methoxycyclohexan-1-ol 3.749, o-Xylene 4.076, 4-Cyclopentene-1,3-dione 4.319 and 1,3,5,7-Cyclooctatetraene 4.585.(Table-3).

The ethanol extract contains about 57 compounds in which Undec-10-ynoic acid, tetradecyl ester having maximum peak percentage area of 24.789 (0.50%), 1,3-Bis(2-pyrazinoxy) propane – 24.298 (0.66), Octadecanoic acid, ethyl ester – 24.119(0.50), Octadecanoic acid-23.978(2.05), 7-Hexadecyn-1-ol - 23.825(19.55), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R*-(E)]]- - 23.341(2.49), 2,6,2',6'-Tetramethylazobenzene-22.968(10.71), Ethyl 14-methyl-hexadecanoate -22.125(2.55), n-Hexadecanoic acid -22.028(12.04) etc.(Table-4).

## DISCUSSION

It is scientifically proved that a plant compound are to be pharmacologically active, it must possess various phytoconstituents which are working together and they may be responsible for different pharmacological properties [Sunday *et al*., 2022]. About 22 phytoconstituents are present in hexane and petroleum ether extracts. Four compounds namely, Neophytadiene, 2(5H)furanone, eugenol and octadecanoic acid are present in ethanol and ethylacetate extract. The compounds namely Dodecane, Dodecane, 2,6,11 – trimethyl and Tetradecanoic acid and Tridecanal are present in Hexane, petroleum ether and ethylacetate extracts. The identified compounds may also use to develop drugs. By conducting phytochemical analysis through GC-MS analysis makes it easier by bringing our more features identified compounds [Velmurugan and Anand 2017]. For example, the compounds Hexadecaionic acid, methanol ester is reported as antioxidant, antiinflamatory and hypochlosterolemic agent [Belakhdar*et al*., 2015].These findings are well in accordance with previous reports [Harura *et al*., 2022, Rajisha and Fernandes., 2020, Khan *et al*., 2016 and Goh., 1995].

These major and common constituents present in hexane and petroleum ether are considered to play a important define machanisms [Salehi *et al*., 2015]. Thus, the identified constituents from *Canscora alata* showed significant medicinal potential of the plant.

## CONCLUSION

The GC-MS analysis of petroleum ether, hexane extracts, ethylacetate and ethanol extracts of *Canscora alata* (Roth) Wall. revealed the presence of different active principles with different pharmacological actions. So, the whole plant extract can be used for treating multiple diseases. However, further studies will require the phytoconstituents and investigating its pharmacological activity and mechanism of action.

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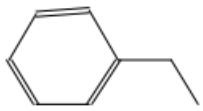
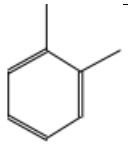
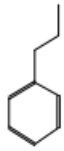
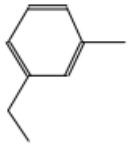
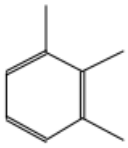
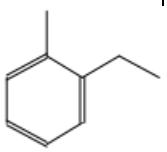
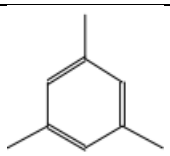
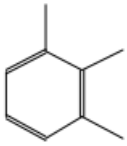
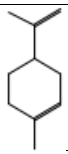
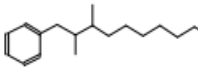
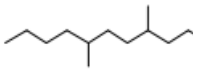
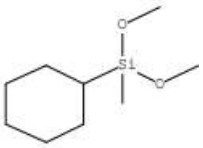
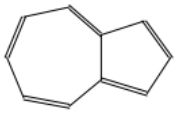
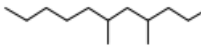
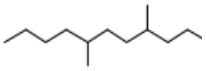
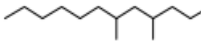
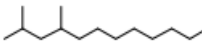
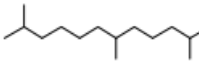
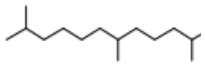
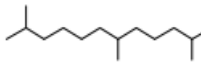
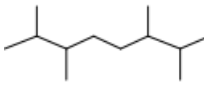
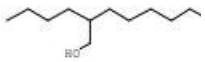
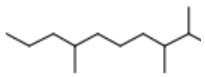
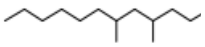
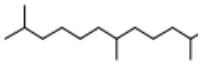
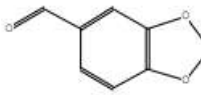
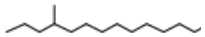
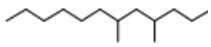
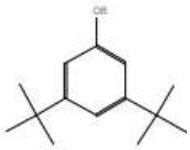
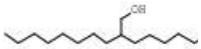
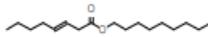
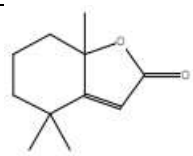
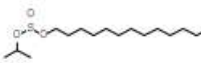
**Table-1 GCMS Analysis of Petroleum ether**

| S.No | R.Time | Name of the compound | Molecular Formula | Molecular Weight | Area % | Hit Spectrum Structure |
|---|---|---|---|---|---|---|
| 1 | 3.669 | Ethylbenzene | $C_8H_{10}$ | 106 | 0.23 |  |
| 2 | 3.856 | o-Xylene | $C_8H_{10}$ | 106 | 0.34 |  |
| 3 | 4.427 | Benzene, 1-azido-2-methyl- | $C_7H_7N_3$ | 133 | 20.25 | |
| 4 | 6.136 | Benzene, propyl- | $C_9H_{12}$ | 120 | 0.26 |  |
| 5 | 6.347 | Benzene, 1-ethyl-3-methyl- | $C_9H_{12}$ | 120 | 1.43 |  |
| 6 | 6.579 | Benzene, 1,2,3-trimethyl- | $C_9H_{12}$ | 120 | 0.55 |  |
| 7 | 6.811 | Benzene, 1-ethyl-2-methyl- | C9H12 | 120 | 0.46 |  |
| 8 | 7.236 | Mesitylene | $C_9H_{12}$ | 120 | 2.70 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | 7.422 | Decane | $C_{10}H_{22}$ | 142 | 0.19 | |
| 10 | 7.909 | Benzene, 1,2,3-trimethyl- | $C_9H_{12}$ | 120 | 0.81 |  |
| 11 | 8.140 | D-Limonene | $C_{10}H_{16}$ | 136 | 0.17 |  |
| 12 | 8.748 | Benzene, (2,3-dimethyldecyl)- | $C_{18}H_{30}$ | 246 | 0.42 |  |
| 13 | 9.739 | Undecane, 4,7-dimethyl- | $C_{13}H_{28}$ | 184 | 0.25 |  |
| 14 | 10.859 | Silane, cyclohexyldimethoxymethyl- | $C_9H_{20}O_2Si$ | 188 | 0.14 |  |
| 15 | 11.424 | Azulene | $C_{10}H_8$ | 128 | 0.10 |  |
| 16 | 11.679 | Dodecane | $C_{12}H_{26}$ | 170 | 0.31 |  |
| 17 | 11.898 | Undecane, 4,6-dimethyl- | $C_{13}H_{28}$ | 184 | 0.18 |  |
| 18 | 12.378 | Tetradecane | $C_{14}H_{30}$ | 198 | 0.09 |  |
| 19 | 12.476 | Undecane, 4,7-dimethyl- | $C_{13}H_{28}$ | 184 | 0.07 |  |
| 20 | 12.584 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.08 |  |
| 21 | 12.736 | 2,4-Dimethyldodecane | $C_{14}H_{30}$ | 198 | 0.18 |  |
| 22 | 12.951 | Dodecane, 2,6,11-trimethyl- | $C_{15}H_{32}$ | 212 | 0.79 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | 13.091 | Dodecane, 2,6,11-trimethyl- | $C_{15}H_{32}$ | 212 | 0.14 |  |
| 24 | 13.183 | Dodecane, 2,6,11-trimethyl- | $C_{15}H_{32}$ | 212 | 0.04 |  |
| 25 | 13.301 | Octane, 2,3,6,7-tetramethyl- | $C_{12}H_{26}$ | 170 | 0.05 |  |
| 26 | 13.361 | 1-Octanol, 2-butyl- | $C_{12}H_{26}O$ | 186 | 0.20 |  |
| 27 | 13.463 | Decane, 2,3,7-trimethyl- | $C_{13}H_{28}$ | 184 | 0.05 |  |
| 28 | 13.692 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.38 |  |
| 29 | 13.842 | Dodecane, 2,6,11-trimethyl- | $C_{15}H_{32}$ | 212 | 0.12 |  |
| 30 | 13.980 | Piperonal | $C_8H_6O_3$ | 150 | 1.24 |  |
| 31 | 14.337 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.12 |  |
| 32 | 14.877 | Tetradecane | $C_{14}H_{30}$ | 198 | 0.29 |  |
| 33 | 15.036 | Pentadecane | $C_{15}H_{32}$ | 212 | 0.27 |  |
| 34 | 15.105 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.16 |  |
| 35 | 15.176 | Tetradecane, 4-methyl- | $C_{15}H_{32}$ | 212 | 0.08 |  |
| 36 | 15.378 | Hexadecane | $C_{16}H_{34}$ | 226 | 0.10 |  |
| 37 | 15.583 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.28 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 38 | 15.674 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.15 |  |
| 39 | 15.765 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.51 |  |
| 40 | 15.862 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.10 |  |
| 41 | 16.120 | Heptadecane | $C_{17}H_{36}$ | 240 | 2.01 |  |
| 42 | 16.216 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.31 |  |
| 43 | 16.392 | Phenol, 3,5-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 206 | 0.88 |  |
| 44 | 16.499 | 1-Decanol, 2-hexyl- | $C_{16}H_{34}O$ | 242 | 0.15 |  |
| 45 | 16.575 | 3-Octenoic acid, nonyl ester | $C_{17}H_{32}O_2$ | 268 | 0.07 |  |
| 46 | 16.729 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.96 |  |
| 47 | 16.797 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | $C_{11}H_{16}O_2$ | 180 | 0.15 |  |
| 48 | 16.865 | Eicosane | $C_{20}H_{42}$ | 282 | 0.08 |  |
| 49 | 16.995 | Eicosane | $C_{20}H_{42}$ | 282 | 0.09 |  |
| 50 | 17.128 | Sulfurous acid, 2-propyl tridecyl ester | $C_{16}H_{34}O_3S$ | 306 | 0.20 |  |
| 51 | 17.209 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.07 |  |

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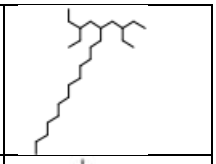
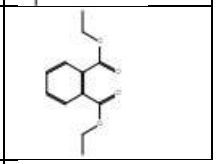
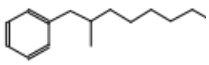
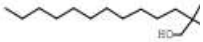
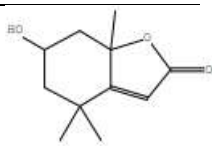
|---|---|---|---|---|---|---|
| 52 | 7.272 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | $C_{26}H_{54}$ | 366 | 0.08 |  |
| 53 | 17.451 | Diethyl Phthalate | $C_{12}H_{14}O_4$ | 222 | 0.25 |  |
| 54 | 17.590 | Hexadecane | $C_{16}H_{34}$ | 226 | 0.39 |  |
| 55 | 17.682 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.26 |  |
| 56 | 17.930 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.59 |  |
| 57 | 18.181 | Octadecane | $C_{18}H_{38}$ | 254 | 0.40 |  |
| 58 | 18.311 | Benzene, (2-methyloctyl)- | $C_{15}H_{24}$ | 204 | 0.16 |  |
| 59 | 18.422 | Hexadecane, 1-iodo- | $C_{16}H_{33}I$ | 352 | 0.05 |  |
| 60 | 18.483 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.31 |  |
| 61 | 18.831 | Eicosane | $C_{20}H_{42}$ | 282 | 1.11 |  |
| 62 | 19.167 | Eicosane | $C_{20}H_{42}$ | 282 | 0.32 |  |
| 63 | 19.350 | Eicosane | $C_{20}H_{42}$ | 282 | 1.11 |  |
| 64 | 19.578 | Tridecanol, 2-ethyl-2-methyl- | $C_{16}H_{34}O$ | 242 | 0.18 |  |
| 65 | 19.756 | 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one | $C_{11}H_{16}O_3$ | 196 | 0.20 |  |

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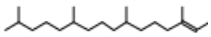
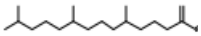
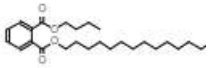
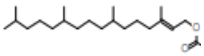
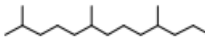
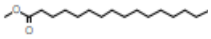
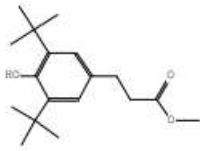
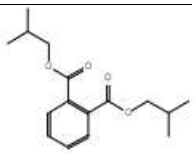
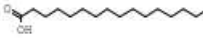
|---|---|---|---|---|---|---|
| 66 | 19.999 | Octadecane | $C_{18}H_{38}$ | 254 | 0.35 |  |
| 67 | 20.207 | Tridecanal | $C_{13}H_{26}O$ | 198 | 0.29 | |
| 68 | 20.341 | 3,7,11,15-Tetramethylhexadec-2-ene | $C_{20}H_{40}$ | 280 | 0.29 |  |
| 69 | 20.417 | Neophytadiene | $C_{20}H_{38}$ | 278 | 3.88 |  |
| 70 | 20.691 | Phthalic acid, butyl tetradecyl ester | $C_{23}H_{36}O_4$ | 376 | 1.57 |  |
| 71 | 20.895 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R*-(E)]]- | $C_{22}H_{42}O_2$ | 338 | 0.84 |  |
| 72 | 20.999 | 2,6,10-Trimethyltridecane | $C_{16}H_{34}$ | 226 | 0.15 |  |
| 73 | 21.056 | Dotriacontane | $C_{32}H_{66}$ | 450 | 0.17 |  |
| 74 | 21.122 | Hexadecane | $C_{16}H_{34}$ | 226 | 0.54 |  |
| 75 | 21.237 | Silane, trichlorooctadecyl- | $C_{18}H_{37}C_{13}Si$ | 386 | 2.75 | |
| 76 | 21.375 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 0.75 |  |
| 77 | 21.461 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | $C_{18}H_{28}O_3$ | 292 | 0.26 |  |
| 78 | 21.548 | Eicosane | $C_{20}H_{42}$ | 282 | 0.19 |  |
| 79 | 21.735 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | $C_{16}H_{22}O_4$ | 278 | 2.23 |  |
| 80 | 21.962 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 12.33 |  |

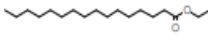
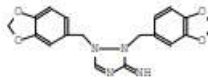
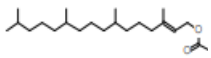
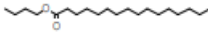
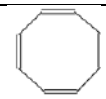
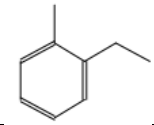
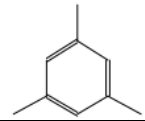
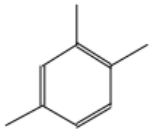
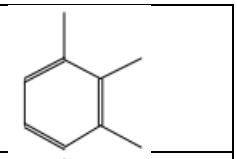
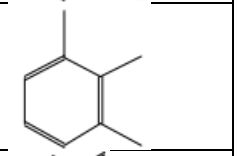
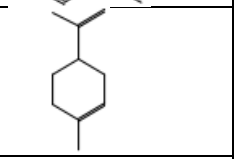
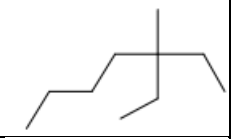
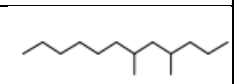
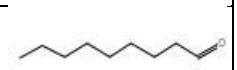
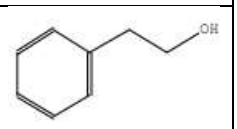
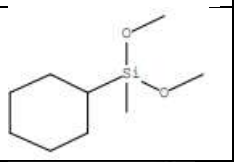
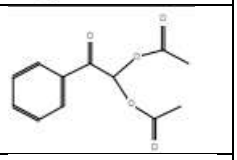
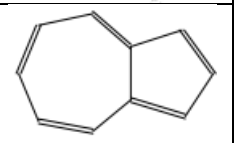
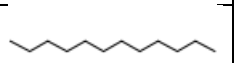
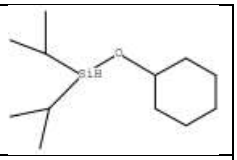
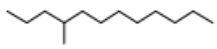
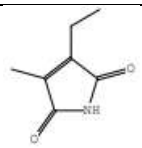
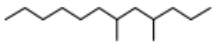
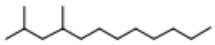
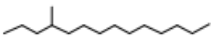
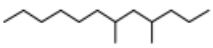
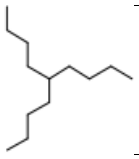
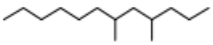
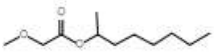
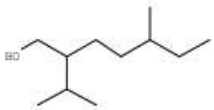
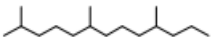
| | | | | | | |
|---|---|---|---|---|---|---|
| 81 | 22.099 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 0.62 |  |
| 82 | 22.184 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.74 |  |
| 83 | 22.709 | 1,2-Dipiperonyl-3-imino-1,2,4-triazine | $C_{18}H_{16}N_4O_4$ | 352 | 2.28 |  |
| 84 | 23.200 | Eicosane | $C_{20}H_{42}$ | 282 | 2.25 |  |
| 85 | 23.330 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R*-(E)]]- | $C_{22}H_{42}O_2$ | 338 | 9.86 |  |
| 86 | 23.425 | Tetrapentacontane | $C_{54}H_{110}$ | 758 | 1.21 |  |
| 87 | 23.737 | Z, Z-8,10-Hexadecadien-1-ol | $C_{16}H_{30}O$ | 238 | 7.70 |  |
| 88 | 24.031 | Hexadecanoic acid, butyl ester | $C_{20}H_{40}O_2$ | 312 | 0.61 |  |
| 89 | 24.179 | Tetracosane | $C_{24}H_{50}$ | 338 | 3.48 |  |

**Table-2 GCMS Analysis of Hexane**

| S.No | R.Time | Name of the compound | Molecular Formula | Molecular Weight | Area % | Hit Spectrum Structure |
|---|---|---|---|---|---|---|
| 1 | 4.361 | 1,3,5,7-Cyclooctatetraene | $C_8H_8$ | 104 | 2.25 |  |
| 2 | 6.348 | Benzene, 1-ethyl-2-methyl- | $C_9H_{12}$ | 120 | 0.07 |  |
| 3 | 6.576 | Mesitylene | $C_9H_{12}$ | 120 | 0.06 |  |
| 4 | 6.810 | Benzene, 1,2,4-trimethyl- | $C_9H_{12}$ | 120 | 0.04 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | 7.231 | Benzene, 1,2,3-trimethyl- | $C_9H_{12}$ | 120 | 0.20 |  |
| 6 | 7.909 | Benzene, 1,2,3-trimethyl- | $C_9H_{12}$ | 120 | 0.04 |  |
| 7 | 8.140 | D-Limonene | $C_{10}H_{16}$ | 136 | 0.16 |  |
| 8 | 8.743 | 3-Ethyl-3-methylheptane | $C_{10}H_{22}$ | 142 | 0.10 |  |
| 9 | 9.736 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.06 |  |
| 10 | 9.816 | Nonanal | $C_9H_{18}O$ | 142 | 0.05 |  |
| 11 | 9.992 | Phenylethyl Alcohol | $C_8H_{10}O$ | 122 | 0.06 |  |
| 12 | 10.860 | Silane, cyclohexyldimethoxymethyl- | $C_9H_{20}O_2Si$ | 188 | 0.13 |  |
| 13 | 11.029 | Phenacylidene diacetate | $C_{12}H_{12}O_5$ | 236 | 0.08 |  |
| 14 | 11.424 | Azulene | $C_{10}H_8$ | 128 | 0.11 |  |
| 15 | 11.681 | Dodecane | $C_{12}H_{26}$ | 170 | 0.18 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16 | 11.852 | 1-Diisopropylsilyloxycyclohexane | $C_{12}H_{26}OSi$ | 214 | 0.14 |  |
| 17 | 12.048 | Dodecane, 4-methyl- | $C_{13}H_{28}$ | 184 | 0.07 |  |
| 18 | 12.238 | 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- | $C_7H_9NO_2$ | 139 | 0.07 |  |
| 19 | 12.380 | Tetradecane | $C_{14}H_{30}$ | 198 | 0.07 |  |
| 20 | 12.475 | Hexadecane | $C_{16}H_{34}$ | 226 | 0.07 |  |
| 21 | 12.583 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.07 |  |
| 22 | 12.737 | 2,4-Dimethyldodecane | $C_{14}H_{30}$ | 198 | 0.15 |  |
| 23 | 12.855 | Tetradecane, 4-methyl- | $C_{15}H_{32}$ | 212 | 0.05 |  |
| 24 | 12.950 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.67 |  |
| 25 | 13.093 | Nonane, 5-butyl- | $C_{13}H_{28}$ | 184 | 0.12 |  |
| 26 | 13.229 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.08 |  |
| 27 | 13.300 | Methoxyacetic acid, 2-octyl ester | $C_{11}H_{22}O_3$ | 202 | 0.06 |  |
| 28 | 13.364 | 2-Isopropyl-5-methyl-1-heptanol | $C_{11}H_{24}O$ | 172 | 0.11 |  |
| 29 | 13.464 | 2,6,10-Trimethyltridecane | $C_{16}H_{34}$ | 226 | 0.11 |  |

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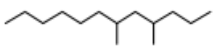
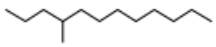
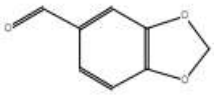
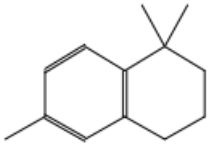
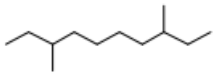
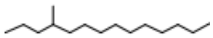
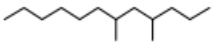
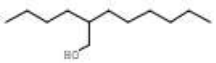
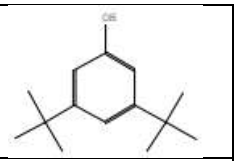
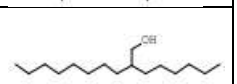
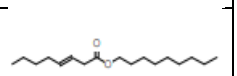
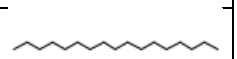
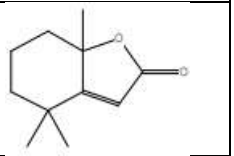
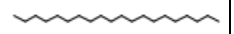
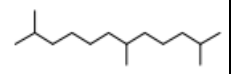
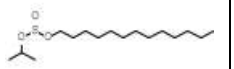
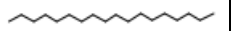
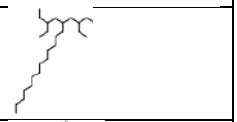
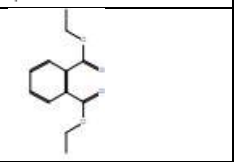
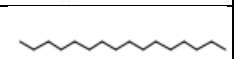
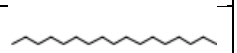
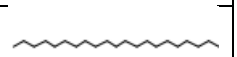
|---|---|---|---|---|---|---|
| 30 | 13.692 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.31 |  |
| 31 | 13.843 | Dodecane, 4-methyl- | $C_{13}H_{28}$ | 184 | 0.08 |  |
| 32 | 13.984 | Piperonal | $C_8H_6O_3$ | 150 | 1.54 |  |
| 33 | 14.303 | Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- | $C_{13}H_{18}$ | 174 | 0.12 |  |
| 34 | 14.452 | Decane, 3,8-dimethyl- | $C_{12}H_{26}$ | 170 | 0.05 |  |
| 35 | 14.878 | Tetradecane | $C_{14}H_{30}$ | 198 | 0.36 |  |
| 36 | 15.106 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.62 |  |
| 37 | 15.177 | Tetradecane, 4-methyl- | $C_{15}H_{32}$ | 212 | 0.05 |  |
| 38 | 15.381 | Hexadecane | $C_{16}H_{34}$ | 226 | 0.08 |  |
| 39 | 15.586 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.25 |  |
| 40 | 15.765 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.90 |  |
| 41 | 15.863 | Dodecane, 4,6-dimethyl- | $C_{14}H_{30}$ | 198 | 0.08 |  |
| 42 | 15.931 | 1-Octanol, 2-butyl- | $C_{12}H_{26}O$ | 186 | 0.04 |  |
| 43 | 16.121 | Heptadecane | $C_{17}H_{36}$ | 240 | 1.74 |  |
| 44 | 16.216 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.29 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 45 | 16.394 | Phenol, 3,5-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 206 | 0.60 |  |
| 46 | 16.500 | 1-Decanol, 2-hexyl- | $C_{16}H_{34}O$ | 242 | 0.13 |  |
| 47 | 16.575 | 3-Octenoic acid, nonyl ester | $C_{17}H_{32}O_2$ | 268 | 0.06 |  |
| 48 | 16.728 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.81 |  |
| 49 | 16.796 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | $C_{11}H_{16}O_2$ | 180 | 0.18 |  |
| 50 | 16.866 | Eicosane | $C_{20}H_{42}$ | 282 | 0.08 |  |
| 51 | 16.995 | Dodecane, 2,6,11-trimethyl- | $C_{15}H_{32}$ | 212 | 0.05 |  |
| 52 | 17.127 | Sulfurous acid, 2-propyl tridecyl ester | $C_{16}H_{34}O_3S$ | 306 | 0.20 |  |
| 53 | 17.207 | Octadecane | $C_{18}H_{38}$ | 254 | 0.05 |  |
| 54 | 17.270 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | $C_{26}H_{54}$ | 366 | 0.08 |  |
| 55 | 17.452 | Diethyl Phthalate | $C_{12}H_{14}O_4$ | 222 | 0.60 |  |
| 56 | 17.592 | Hexadecane | $C_{16}H_{34}$ | 226 | 0.31 |  |
| 57 | 17.682 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.22 |  |
| 58 | 17.930 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.48 |  |

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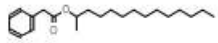
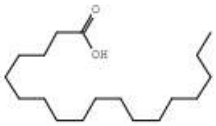
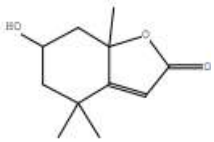
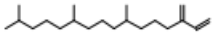
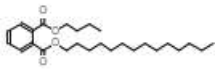
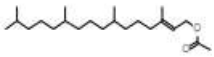
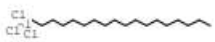
|---|---|---|---|---|---|---|
| 59 | 18.311 | Benzeneacetic acid, 2-tetradecyl ester | $C_{22}H_{36}O_2$ | 332 | 0.19 |  |
| 60 | 18.485 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.24 |  |
| 61 | 18.832 | Eicosane | $C_{20}H_{42}$ | 282 | 1.24 |  |
| 62 | 19.166 | Nonyl tetradecyl ether | $C_{23}H_{48}O$ | 340 | 0.28 |  |
| 63 | 19.351 | Eicosane | $C_{20}H_{42}$ | 282 | 0.98 |  |
| 64 | 19.605 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 0.40 |  |
| 65 | 19.762 | 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one | $C_{11}H_{16}O_3$ | 196 | 0.24 |  |
| 66 | 20.000 | Octadecane | $C_{18}H_{38}$ | 254 | 0.30 |  |
| 67 | 20.208 | Tridecanal | $C_{13}H_{26}O$ | 198 | 0.27 | |
| 68 | 20.420 | Neophytadiene | $C_{20}H_{38}$ | 278 | 4.37 |  |
| 69 | 20.691 | Phthalic acid, butyl tetradecyl ester | $C_{26}H_{42}O_4$ | 418 | 1.35 |  |
| 70 | 20.896 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R*-(E)]]- | $C_{22}H_{42}O_2$ | 338 | 1.15 |  |
| 71 | 21.059 | Eicosane | $C_{20}H_{42}$ | 282 | 0.13 |  |
| 72 | 21.124 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.51 |  |
| 73 | 21.240 | Silane, trichlorooctadecyl- | $C_{18}H_{37}Cl_3Si$ | 386 | 2.48 |  |

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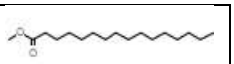
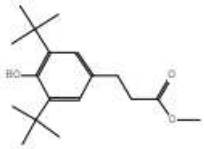
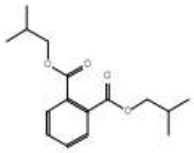
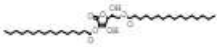
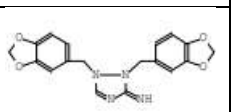
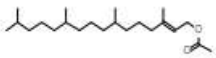
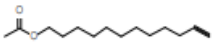
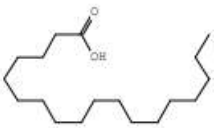
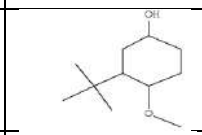
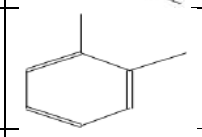
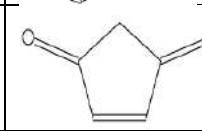
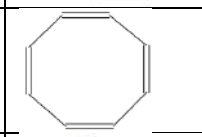
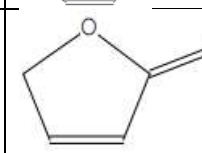
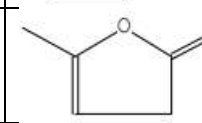
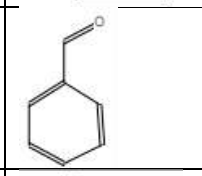
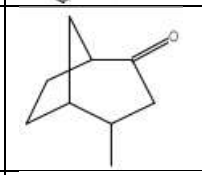
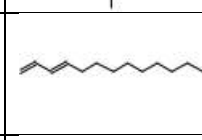
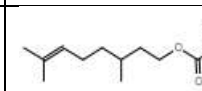
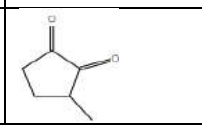
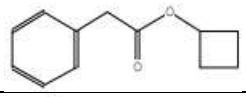
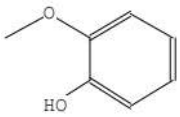
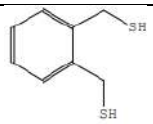
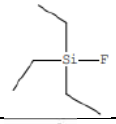
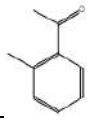
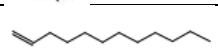
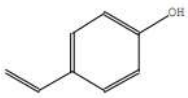
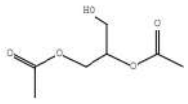
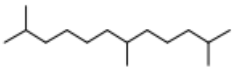
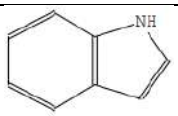
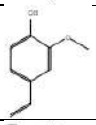
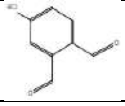
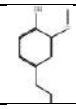
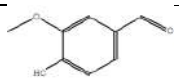
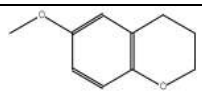
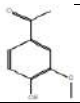
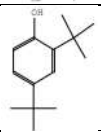
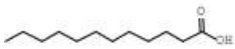
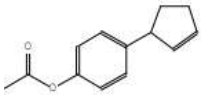
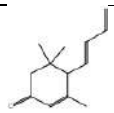
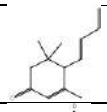
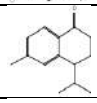
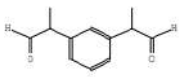
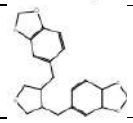
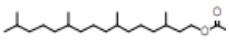
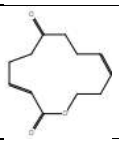
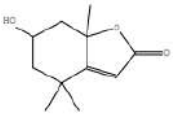
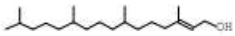
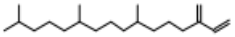
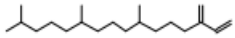
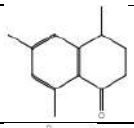
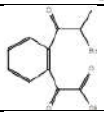
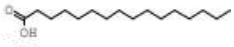
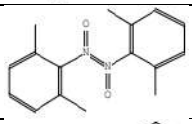
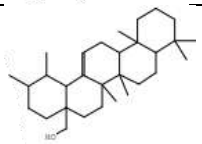
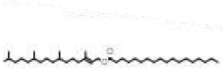
|---|---|---|---|---|---|---|
| 74 | 21.376 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 0.61 |  |
| 75 | 21.463 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | $C_{18}H_{28}O_3$ | 292 | 0.23 |  |
| 76 | 21.556 | Pentatriacontane | $C_{35}H_{72}$ | 492 | 0.17 |  |
| 77 | 21.632 | Heneicosane | $C_{21}H_{44}$ | 296 | 0.09 |  |
| 78 | 21.739 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | $C_{16}H_{22}O_4$ | 278 | 2.36 |  |
| 79 | 22.009 | l-(+)-Ascorbic acid 2,6-dihexadecanoate | $C_{38}H_{68}O_8$ | 652 | 19.55 |  |
| 80 | 22.721 | 1,2-Dipiperonyl-3-imino-1,2,4-triazine | $C_{18}H_{16}N_4O_4$ | 352 | 2.47 |  |
| 81 | 23.204 | Eicosane | $C_{20}H_{42}$ | 282 | 2.62 |  |
| 82 | 23.341 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- | $C_{22}H_{42}O_2$ | 338 | 10.39 |  |
| 83 | 23.430 | Tetrapentacontane | $C_{54}H_{110}$ | 758 | 0.85 |  |
| 84 | 23.790 | 11-Dodecyn-1-ol acetate | $C_{14}H_{24}O_2$ | 224 | 24.37 |  |
| 85 | 23.949 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 2.15 |  |
| 86 | 24.188 | Tetrapentacontane | $C_{54}H_{110}$ | 758 | 3.84 |  |
| 87 | 24.765 | Z, Z-6,28-Heptatriactontadien-2-one | $C_{37}H_{70}O$ | 530 | 0.36 |  |

**Table -3 GCMS Analysis of Ethyl Acetate**

| S.No | RT | Name of the Compound | Molecular Formula | Molecular Weight | Area % | Hit spectrum |
|---|---|---|---|---|---|---|
| 1 | 3.749 | 3, Trans-(1,1-dimethylethyl)-4, cis-methoxycyclohexan-1-ol | $C_{11}H_{22}O_2$ | 186 | 0.27 |  |
| 2 | 4.076 | o-Xylene | $C_8H_{10}$ | 106 | 0.19 |  |
| 3 | 4.319 | 4-Cyclopentene-1,3-dione | $C_5H_4O_2$ | 96 | 0.11 |  |
| 4 | 4.585 | 1,3,5,7-Cyclooctatetraene | $C_8H_8$ | 104 | 0.25 |  |
| 5 | 5.057 | 2(5H)-Furanone | $C_4H_4O_2$ | 84 | 0.23 |  |
| 6 | 5.536 | 2(3H)-Furanone, 5-methyl- | $C_5H_6O_2$ | 98 | 0.52 |  |
| 7 | 6.514 | Benzaldehyde | $C_7H_6O$ | 106 | 0.09 |  |
| 8 | 7.106 | 4-Methyl-bicyclo [3.2.1] octan-2-one | $C_9H_{14}O$ | 138 | 0.17 |  |
| 9 | 7.268 | E-11,13-Tetradecadien-1-ol | $C_{14}H_{26}O$ | 210 | 0.07 |  |
| 10 | 7.456 | . beta. -Citronellol, chlorodifluoroacetate | $C_{12}H_{19}ClF_2O_2$ | 268 | 0.12 |  |
| 11 | 8.194 | 1,2-Cyclopentanedione, 3-methyl- | $C_6H_8O_2$ | 112 | 0.14 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 | 8.537 | Phenylacetic acid, cyclobutyl ester | $C_{12}H_{14}O_2$ | 190 | 0.44 |  |
| 13 | 9.476 | Phenol, 2-methoxy- | $C_7H_8O_2$ | 124 | 0.76 |  |
| 14 | 10.056 | o-Xylene-.alpha.,.alpha.'-dithio | $C_8H_{10}S_2$ | 170 | 0.22 |  |
| 15 | 11.054 | Silane, triethylfluoro- | $C_6H_{15}FSi$ | 134 | 0.22 |  |
| 16 | 11.357 | 6-Methylheptanoic acid | $C_8H_{16}O_2$ | 144 | 0.06 | |
| 17 | 11.446 | Ethanone, 1-(2-methylphenyl)- | $C_9H_{10}O$ | 134 | 0.16 |  |
| 18 | 11.525 | 1-Dodecene | $C_{12}H_{24}$ | 168 | 0.13 |  |
| 19 | 11.687 | Dodecane | $C_{12}H_{26}$ | 170 | 0.04 | |
| 20 | 12.123 | 4-Vinylphenol | $C_8H_8O$ | 120 | 1.89 |  |
| 21 | 12.499 | Glycerol 1,2-diacetate | $C_7H_{12}O_5$ | 176 | 0.33 |  |
| 22 | 12.952 | Dodecane, 2,6,11-trimethyl- | $C_{15}H_{32}$ | 212 | 0.12 |  |
| 23 | 13.341 | Indole | $C_8H_7N$ | 117 | 0.14 |  |
| 24 | 13.603 | 2-Methoxy-4-vinylphenol | $C_9H_{10}O_2$ | 150 | 1.50 |  |
| 25 | 14.073 | 2,3-Diformylphenol | $C_8H_6O_3$ | 150 | 7.82 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 26 | 14.226 | Eugenol | $C_{10}H_{12}O_2$ | 164 | 0.12 |  |
| 27 | 14.760 | 1-Hexadecanol | $C_{16}H_{34}O$ | 242 | 0.45 |  |
| 28 | 14.935 | Vanillin | $C_8H_8O_3$ | 152 | 0.23 |  |
| 29 | 15.624 | 6-Chromanol, methyl ether | $C_{10}H_{12}O_2$ | 164 | 0.37 |  |
| 30 | 16.145 | Apocynin | $C_9H_{10}O_3$ | 166 | 0.60 |  |
| 31 | 16.402 | 2,4-Di-tert-butylphenol | $C_{14}H_{22}O$ | 206 | 0.33 |  |
| 32 | 17.141 | Dodecanoic acid | $C_{12}H_{24}O_2$ | 200 | 0.33 |  |
| 33 | 17.260 | 4-(Cyclopent-2-en-1-yl) phenol, acetate | $C_{13}H_{14}O_2$ | 202 | 0.13 |  |
| 34 | 17.491 | 1-Heptadecene | $C_{17}H_{34}$ | 238 | 0.69 |  |
| 35 | 17.839 | Megastigmatrienone | $C_{13}H_{18}O$ | 190 | 0.07 |  |
| 36 | 18.000 | Megastigmatrienone | $C_{13}H_{18}O$ | 190 | 0.26 |  |
| 37 | 18.153 | 4-Isopropyl-6-methyl-1-tetralone | $C_{14}H_{18}O$ | 202 | 0.27 |  |
| 38 | 18.253 | Benzene, 1,3-bis(1-formylethyl)- | $C_{12}H_{14}O_2$ | 190 | 0.07 |  |
| 39 | 18.461 | Deoxycubebin | $C_{20}H_{20}O_5$ | 340 | 0.84 |  |
| 40 | 18.830 | Tridecanal | $C_{13}H_{26}O$ | 198 | 0.70 | |
| 41 | 19.020 | Pentadecanal- | $C_{15}H_{30}O$ | 226 | 0.21 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 42 | 19.156 | Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester | $C_{22}H_{44}O_2$ | 340 | 0.09 |  |
| 43 | 19.366 | (3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione | $C_{12}H_{16}O_3$ | 208 | 0.53 |  |
| 44 | 19.605 | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 228 | 0.79 | |
| 45 | 19.845 | 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one | $C_{11}H_{16}O_3$ | 196 | 1.03 |  |
| 46 | 20.210 | Tetradecanal | $C_{14}H_{28}O$ | 212 | 0.09 | |
| 47 | 20.437 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 9.83 |  |
| 48 | 20.691 | Neophytadiene | $C_{20}H_{38}$ | 278 | 1.60 |  |
| 49 | 20.901 | Neophytadiene | $C_{20}H_{38}$ | 278 | 2.48 |  |
| 50 | 21.022 | 1(2H)-Naphthalenone, 3,4-dihydro-4,6,8-trimethyl- | $C_{13}H_{16}O$ | 188 | 0.32 |  |
| 51 | 21.144 | 2-(2-Bromopropionyl) phenylglyoxylic acid | $C_{11}H_9BrO_4$ | 284 | 0.46 |  |
| 52 | 21.989 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 18.24 |  |
| 53 | 22.868 | 2,6,2',6'-Tetramethylazobenzene | $C_{16}H_{18}N_2O_2$ | 270 | 15.13 |  |
| 54 | 23.194 | Urs-12-en-28-ol | $C_{30}H_{50}O$ | 426 | 0.57 |  |
| 55 | 23.354 | Phytyl stearate | $C_{38}H_{74}O_2$ | 562 | 12.03 |  |

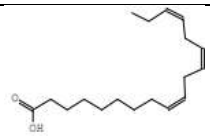
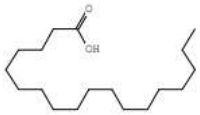
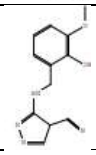
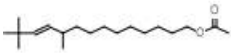
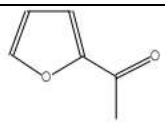
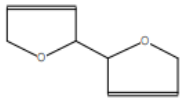
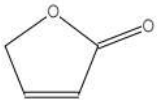
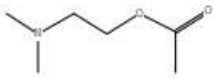
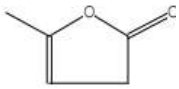
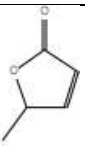
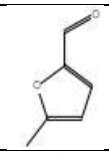
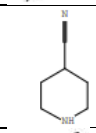
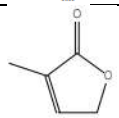
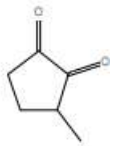
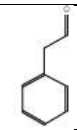
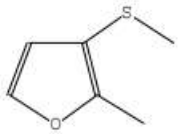
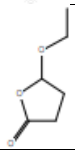
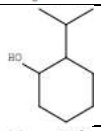
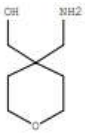
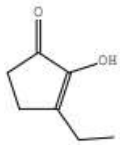
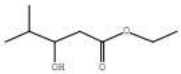
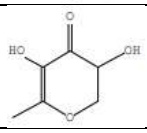
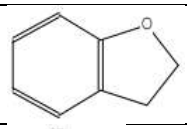
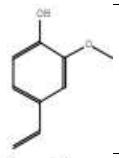
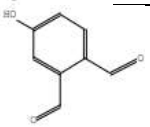
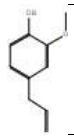
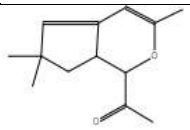
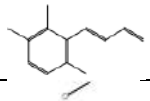
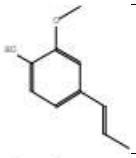
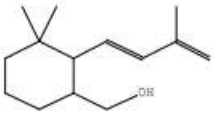
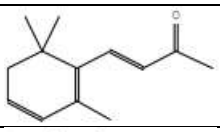
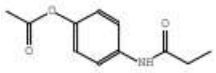
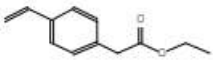
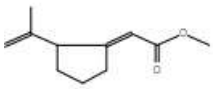
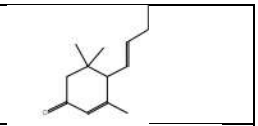
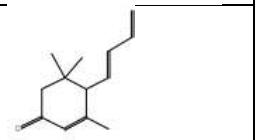
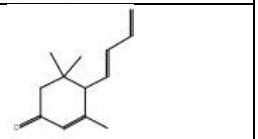
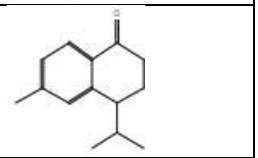
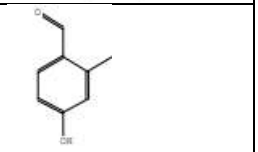
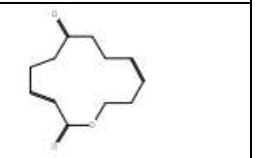
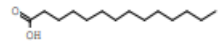
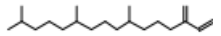
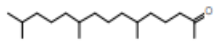
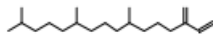
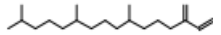
| | | | | | | |
|---|---|---|---|---|---|---|
| 56 | 23.756 | 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- | $C_{18}H_{30}O_2$ | 278 | 12.48 |  |
| 57 | 23.929 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 1.71 |  |
| 58 | 24.193 | 4H-Pyrazole-4-carbonitrile, 3-[[(2-hydroxy-3-methoxyphenyl) methyl] amino]- | $C_{12}H_{12}N_4O_2$ | 244 | 0.36 |  |
| 59 | 24.766 | E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate | $C_{19}H_{36}O_2$ | 296 | 0.59 |  |

**Table-4 GCMS ANALYSIS OF ETHANOL**

| S.NO | R.Time | Name of the Compound | Molecular Formula | Molecular Weight | Area % | Hit Spectrum Structure |
|---|---|---|---|---|---|---|
| 1 | 4.436 | Levoglucosenone | $C_6H_6O_3$ | 126 | 4.65 | |
| 2 | 4.922 | 4-Cyclopentene-1,3-dione | $C_5H_4O_2$ | 96 | 0.06 | |
| 3 | 5.147 | Ethanone, 1-(2-furanyl)- | $C_6H_6O_2$ | 110 | 0.08 |  |
| 4 | 5.472 | 2,2'-Bifuran, 2,2',5,5'-tetrahydro- | $C_8H_{10}O_2$ | 138 | 0.40 |  |
| 5 | 5.790 | 2(5H)-Furanone | $C_4H_4O_2$ | 84 | 1.33 |  |
| 6 | 5.910 | Acetic acid, 2-(dimethylamino)ethyl ester | $C_6H_{13}NO_2$ | 131 | 0.57 |  |
| 7 | 6.169 | 2(3H)-Furanone, 5-methyl- | $C_5H_6O_2$ | 98 | 0.66 |  |
| 8 | 6.303 | 2(5H)-Furanone, 5-methyl- | $C_5H_6O_2$ | 98 | 0.23 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | 6.579 | 2-Furancarboxaldehyde, 5-methyl- | $C_6H_6O_2$ | 110 | 1.26 |  |
| 10 | 6.866 | Piperidine-4-carbonitrile | $C_6H_{10}N_2$ | 110 | 0.57 |  |
| 11 | 7.174 | 2(5H)-Furanone, 3-methyl- | $C_5H_6O_2$ | 98 | 0.14 |  |
| 12 | 7.729 | 1,2-Cyclopentanedione, 3-methyl- | $C_6H_8O_2$ | 112 | 0.28 |  |
| 13 | 8.580 | Benzeneacetaldehyde | $C_8H_8O$ | 120 | 1.95 |  |
| 14 | 8.885 | 2-Methyl-3-(methylthio) furan | $C_6H_8OS$ | 128 | 1.71 |  |
| 15 | 9.149 | 2(3H)-Furanone, 5-ethoxydihydro- | $C_6H_{10}O_3$ | 130 | 0.13 |  |
| 16 | 9.686 | Cyclohexanol, 2-(1-methylethyl)- | $C_9H_{18}O$ | 142 | 6.68 |  |
| 17 | 10.140 | [4-(Aminomethyl) oxan-4-yl] methanol | $C_7H_{15}NO_2$ | 145 | 0.26 |  |
| 18 | 10.326 | 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy- | $C_7H_{10}O_2$ | 126 | 0.73 |  |
| 19 | 10.727 | Pentanoic acid, 3-hydroxy-4-methyl-, ethyl ester | $C_8H_{16}O_3$ | 160 | 1.49 |  |
| 20 | 11.410 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | $C_6H_8O_4$ | 144 | 0.35 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 21 | 12.305 | Benzofuran, 2,3-dihydro- | $C_8H_8O$ | 120 | 3.30 |  |
| 22 | 13.702 | 2-Methoxy-4-vinylphenol | $C_9H_{10}O_2$ | 150 | 3.94 |  |
| 23 | 14.148 | 2,3-Diformylpheno | $C_8H_6O_3$ | 150 | 5.58 |  |
| 24 | 14.276 | Eugenol | $C_{10}H_{12}O_2$ | 164 | 0.54 |  |
| 25 | 14.765 | 1-Tetradecene | $C_{14}H_{28}$ | 196 | 0.48 | |
| 26 | 14.889 | 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone | $C_{13}H_{18}O_2$ | 206 | 0.48 |  |
| 27 | 15.042 | (E)-1-(2,3,6-trimethylphenyl) buta-1,3-diene (TPB, 1) | $C_{13}H_{16}$ | 172 | 0.46 |  |
| 28 | 15.667 | trans-Isoeugenol | $C_{10}H_{12}O_2$ | 164 | 0.36 |  |
| 29 | 15.937 | Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-methyl-1,3-butadienyl)- | $C_{14}H_{24}O$ | 208 | 0.93 |  |
| 30 | 16.068 | 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-3-en-2-one | $C_{13}H_{18}O$ | 190 | 0.33 |  |
| 31 | 16.434 | N-(4-Hydroxyphenyl) propanamide, O-acetyl- | $C_{11}H_{13}NO_3$ | 207 | 0.36 |  |
| 32 | 16.812 | Benzeneacetic acid, 4-ethenyl-, ethyl ester | $C_{12}H_{14}O_2$ | 190 | 0.26 |  |
| 33 | 17.192 | Acetic acid, (2-isopropenylcyclopentylidene)-, methyl ester | $C_{11}H_{16}O_2$ | 180 | 0.49 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 34 | 17.438 | Megastigmatrienone | $C_{13}H_{18}O$ | 190 | 0.50 |  |
| 35 | 17.500 | 1-Hexadecanol | $C_{16}H_{34}O$ | 242 | 0.38 |  |
| 36 | 17.876 | Megastigmatrienone | $C_{13}H_{18}O$ | 190 | 0.15 |  |
| 37 | 18.046 | Megastigmatrienone | $C_{13}H_{18}O$ | 190 | 0.74 |  |
| 38 | 18.181 | 4-Isopropyl-6-methyl-1-tetralone | $C_{14}H_{18}O$ | 202 | 0.16 |  |
| 39 | 18.486 | 4-Hydroxy-2-methylbenzaldehyde | $C_8H_8O_2$ | 136 | 0.41 |  |
| 40 | 19.421 | (3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione | $C_{12}H_{16}O_3$ | 208 | 0.62 |  |
| 41 | 19.636 | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 228 | 0.55 |  |
| 42 | 19.924 | 1-Nonadecene | $C_{19}H_{38}$ | 266 | 0.32 |  |
| 43 | 20.424 | Neophytadiene | $C_{20}H_{38}$ | 278 | 2.24 |  |
| 44 | 20.489 | 2-Pentadecanone, 6,10,14-trimethyl- | $C_{18}H_{36}O$ | 268 | 0.28 |  |
| 45 | 20.692 | Neophytadiene | $C_{20}H_{38}$ | 278 | 0.46 |  |
| 46 | 20.901 | Neophytadiene | $C_{20}H_{38}$ | 278 | 0.70 |  |

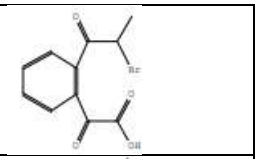
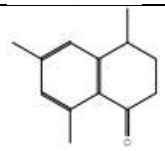
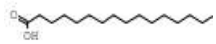
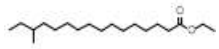
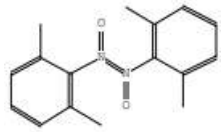
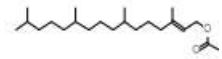
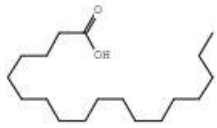
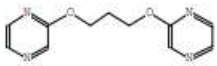
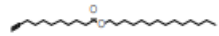
| | | | | | | |
|---|---|---|---|---|---|---|
| 47 | 21.065 | 2-(2-Bromopropionyl) phenylglyoxylic acid | $C_{11}H_9BrO_4$ | 284 | 0.28 |  |
| 48 | 21.190 | 1(2H)-Naphthalenone, 3,4-dihydro-4,6,8-trimethyl- | $C_{13}H_{16}O$ | 188 | 0.39 |  |
| 49 | 22.028 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 12.04 |  |
| 50 | 22.125 | Ethyl 14-methyl-hexadecanoate | $C_{19}H_{38}O_2$ | 298 | 2.55 |  |
| 51 | 22.968 | 2,6,2',6'-Tetramethylazobenzene | $C_{16}H_{18}N_2O_2$ | 270 | 10.71 |  |
| 52 | 23.341 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R*-(E)]]- | $C_{22}H_{42}O_2$ | 338 | 2.49 |  |
| 53 | 23.825 | 7-Hexadecyn-1-ol | $C_{16}H_{30}O$ | 238 | 19.55 | |
| 54 | 2.05 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 2.05 |  |
| 55 | 24.119 | Octadecanoic acid, ethyl ester | $C_{20}H_{40}O_2$ | 312 | 0.50 | |
| 56 | 24.298 | 1,3-Bis(2-pyrazinoxy) propane | $C_{11}H_{12}N_4O_2$ | 232 | 0.66 |  |
| 57 | 24.789 | Undec-10-ynoic acid, tetradecyl ester | $C_{25}H_{46}O_2$ | 378 | 0.50 |  |

Plate - 1

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**RESEARCH ARTICLE**

# On Solving Neutrosophic Linear and Nonlinear as a Neutrosophic Fuzzy Linear Complementarity Problem

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**ABSTRACT**

The paper proposes a new approach to solve the Neutrosophic Quadratic Programming Problem(NQPP). Here, single valued triangular neutrosophic number replaces all fuzzy coefficients in the objective functions and constraints. We formulate other existing programming problems, such as the linear programming problems and the quadratic programming problems, into the linear complementarity programming problems and hence solve them using a solution algorithm for the LCP. Real-life applications taken from Linear Programming Problems(LPP) and Quadratic Programming Problems(QPP) are then used to demonstrate the elegance, precision and utility of these complementarity programming problems.

**Keywords:** Fuzzy Quadratic programming problem, Fuzzy Linear Complementarity problem, Single valued Triangular Neutrosophic Number, Lemke's method

## INTRODUCTION

Smarandache[5,6], which is a generalization of classical sets, fuzzy sets, intuitionistic fuzzy sets, etc., first introduced the concept of Neutrosophic Set(NS). The classical fuzzy set concept proposed by Zadeh[1] is a very effective mathematical tool for handling the complexity that generally arises from uncertainty and ambiguity in real-world scenarios. Actually, it's much more difficult to use many linear programming techniques. Furthermore, the non linear programming model cannot be representative of a large number of real problems. As a result of this, efforts have

been done to establish a more general collection of mathematical programming methods, and significant progress in the field of fuzzy quadratic programming problems has been achieved. Suggested pairings of inequality restrictions must be complementary, meaning that at least one must hold with equality for a variety of physical and economic developments to be most naturally illustrated. The objective function may, in principle, be attached to such conditions but is more often used for the purpose of establishing complementarity problems where a realistic solution can be found. Actually, complementarity is able to considered a specific case of optimization. For both linear and nonlinear optimization, the usual optimality standards are complementarity problems with the environment. Linear Complementarity Problems can be applied in numerous fields of optimization. An algorithm aimed at dealing with a linear complementarity problem and a quadratic programming problem, for instance, can be utilized for defining a linear programming problem as a linear complementarity problem (LCP). In that case, the Lemke algorithm can be used when solving quadratic programming problems using KKT conditions as a LCP. In this research, an innovative approach to fuzzy neutrosophic quadratic programming and fuzzy neutrosophic linear programming problems is presented. These problems can be addressed by transforming them into fuzzy neutrosophic linear complementarity problems. Compared to fuzzy sets and intuitionistic fuzzy sets, neutrosophic sets are more relevant and helpful since they consistently express truth, ambiguity, and untruth. Linear Complementarity Problems Applications to other Programming Problems[10] , Wang et al. established neutrosophic sets, which have been expanded to single valued neutrosophic sets[7,8]. The following is the order in which the paper is organized. The single valued triangular neutrosophic number (SVTN) is specified in Section 2, as are the fuzzy arithmetical operators. Section 3 describes a technique for solving the fuzzy linear complementarity problem in neutrosophic. Section 4 discusses the proposed method of NFLPP and NFQPP, which is to be referred to as an NFLCP. In conclusion, a case study demonstrates the effectiveness of the proposed method in Section 5.

**Preliminaries**

2.1) Definition: Consider the universe E. We define the Neutrosophic set A over E as follows: $A = \{\langle x, (\tau_{\tilde{A}}(x),$ *iAx, ωAx :x∈E,* While the terms truth , inaccuracy and uncertainty involve *τAx, iAx, ωAx,* respectively. They are respectively defined by $\tau_{\tilde{A}} : E \to ]^{-}0, 1^{+}[$, $i_{\tilde{A}} : E \to ]^{-}0, 1^{+}[$, $\omega_{\tilde{A}} : E \to ]^{-}0, 1^{+}[$ , Such that $0^{-} \leq \tau_{\tilde{A}}(x) + i_{\tilde{A}}(x) + \omega_{\tilde{A}}(x) \leq 3^{+}$

2.2) Definition: If the value of $\tilde{A} = \langle (a, b, c); \tau_{\tilde{a}}, i_{\tilde{a}}, \omega_{\tilde{a}} \rangle$ describe a Single Valued Triangular Neutrosophic Number (SVTNN). whose three membership functions for the truth, indeterminacy and a falsity of X are given by

$$\tau_{\tilde{A}}(x) = \begin{cases} \frac{(x-a)\tau_{\tilde{a}}}{(b-a)} & ,a \leq x \leq b \\ \tau_{\tilde{a}} & ,x = b \\ \frac{(c-x)\tau_{\tilde{a}}}{(c-b)} & ,b \leq x \leq c \\ 0 & ,otherwise \end{cases},$$

$$i_{\tilde{A}}(x) = \begin{cases} \frac{(b-x)i_{\tilde{a}}}{(b-a)} & ,a \leq x \leq b \\ i_{\tilde{a}} & ,x = b \\ \frac{(x-c)i_{\tilde{a}}}{(c-b)} & ,b \leq x \leq c \\ 1 & ,otherwise \end{cases},$$

$$\omega_{\tilde{A}}(x) = \begin{cases} \frac{(b-x)\omega_{\tilde{a}}}{(b-a)} & ,a \le x \le b \\ \omega_{\tilde{a}} & ,x = b \\ \frac{(x-c)\omega_{\tilde{a}}}{(c-b)} & ,b \le x \le c \\ 1 & ,otherwise \end{cases}$$

Where $0 \le \tau_{\tilde{A}}(x) + i_{\tilde{A}}(x) + \omega_{\tilde{A}}(x) \le 3, x \epsilon \tilde{A}$ ,

Additionally, when $a > 0$, $\tilde{A}$ is called a non-negative SVTNN.

Similarly, when $a < 0$, $\tilde{A}$ becomes a negative SVTNN

*Arithmetic Operations on Single Valued Triangular Neutrosophic Numbers:*

Let $\tilde{a} = \langle (a_1, b_1, c_1);\ \tau_{\tilde{a}}, i_{\tilde{a}}, \omega_{\tilde{a}} \rangle$ and $\tilde{b} = \langle (a_2, b_2, c_2);\ \tau_{\tilde{b}}, i_{\tilde{b}}, \omega_{\tilde{b}} \rangle$ be two single valued triangular neutrosophic numbers and $\gamma \neq 0$.

$$\tilde{a} + \tilde{b} = \langle (a_1 + a_2, b_1 + b_2, c_1 + c_2);\ \tau_{\tilde{a}} \wedge \tau_{\tilde{b}}, i_{\tilde{a}} \vee i_{\tilde{b}}, \omega_{\tilde{a}} \vee \omega_{\tilde{b}} \rangle$$

$$\tilde{a} - \tilde{b} = \langle (a_1 - c_2, b_1 - b_2, c_1 - a_2);\ \tau_{\tilde{a}} \wedge \tau_{\tilde{b}}, i_{\tilde{a}} \vee i_{\tilde{b}}, \omega_{\tilde{a}} \vee \omega_{\tilde{b}} \rangle$$

$$\tilde{a}\tilde{b} = \{Min(a_1a_2, a_1c_2, c_1a_2, c_1c_2), b_1b_2, Max(a_1a_2, a_1c_2, c_1a_2, c_1c_2);\ \tau_{\tilde{a}} \wedge \tau_{\tilde{b}}, i_{\tilde{a}} \vee i_{\tilde{b}}, \omega_{\tilde{a}} \vee \omega_{\tilde{b}}\}$$

$$\tilde{a} / \tilde{b} = \{Min(a_1/a_2, a_1/c_2, c_1/a_2, c_1/c_2), b_1/b_2, Max(a_1/a_2, a_1/c_2, c_1/a_2, c_1/c_2);\ \tau_{\tilde{a}} \wedge \tau_{\tilde{b}}, i_{\tilde{a}} \vee i_{\tilde{b}}, \omega_{\tilde{a}} \vee \omega_{\tilde{b}}\}$$

$$\gamma\tilde{a} = \begin{cases} \langle (\gamma a_1, \gamma b_1, \gamma c_1);\ \tau_{\tilde{a}}, i_{\tilde{a}}, \omega_{\tilde{a}} \rangle, (\gamma > 0) \\ \langle (\gamma c_1, \gamma b_1, \gamma a_1);\ \tau_{\tilde{a}}, i_{\tilde{a}}, \omega_{\tilde{a}} \rangle, (\gamma < 0) \end{cases}$$

Fuzzy Linear Programming Problem as a Fuzzy Linear Complementarity Problem (FLCP)

*Fuzzy Linear Complementarity Problem (FLCP)*

The linear complementarity problem is to determine $\tilde{w} \in \mathbb{R}^n$ and $\tilde{z} \in \mathbb{R}^n$ such that the following three conditions satisfy, given a matrix $\tilde{M} \in \mathbb{R}^{n \times n}$ and a vector $\tilde{q} \in \mathbb{R}^n$.

$\tilde{W} - \tilde{M}\tilde{Z} = \tilde{q}$ (1)

$\tilde{w} \ge 0, \tilde{z} \ge 0, j = 1,2,3, \ldots\ldots n$ (2)

$\tilde{w}^T \tilde{z} = 0$ (3)

A pair of fuzzy linear complementary variables is denoted as follows $(\tilde{W}_j, \tilde{Z}_j)$.

3.2) *Definition:*

The system above has a solution $(\tilde{W}, \tilde{Z})$ where $\tilde{w} = (\tilde{w}_1, \tilde{w}_2, \tilde{w}_3, \ldots)$ *and* $\tilde{z} = (\tilde{z}_1, \tilde{z}_2, \tilde{z}_3, \ldots)$. It may be derived from (2) and (3) that for any j = 1, 2, 3,.. n, $\tilde{w}_j \ge 0$ and $\tilde{z}_j \ge 0$ Since $\tilde{W}_j\tilde{Z}_j = 0$. The response to this problem is $(\tilde{w}, \tilde{z}) = (\tilde{w}_1, \tilde{w}_2, \tilde{w}_3, \ldots \tilde{w}_n, \tilde{z}_1, \tilde{z}_2, \tilde{z}_3, \ldots \tilde{z}_n)$ if $(\tilde{W}, \tilde{Z})$ is a Neutrosophic basic feasible solution to (1) and (2) with one of the pair $(\tilde{W}_j, \tilde{Z}_j)$ basic for each j =1, 2, 3,…n. The concept of "Neutrosophic complementary feasible solution" applies to this problem.

*Linear Complementarity Problem Formulation – Relationship between LPP and LCP*

The symmetric form of the primal problem can be used to express a general linear programming problem (LPP) as follows.

$Minimize\ z = CX$

Subject to $AX \ge b$ → (4)

$x \ge 0$

Assume that matrix A has an order of $m \times n$. If the optimal solution to the essential problem (4) is illustrated by $x \epsilon \mathbb{R}^n$, then the dual problem of (4) symmetrically becomes the Dual problem.

$Maximize\ z = b^T Y$

Subject to $A^TY \geq C^T$ → (5)
$Y \geq 0$

By the well know duality theory of LPP, there exists a dual vector $y \epsilon \mathbb{R}^m$ which is an optimal solution to problem (5). By introducing slack dual variable $u \epsilon \mathbb{R}^m$ it follows that the constraint of (5) becomes,
$u - (-A^TY) = C^T$ → (6)
For (4), we introduce the primal surplus variable $v \epsilon \mathbb{R}^n$, then the constraint of problem (4) now follows;
$v - AX = -b$ → (7)
We then combine the constraints of (6) and (7) in matrix form.

$$\begin{pmatrix} u \\ \vdots \\ v \end{pmatrix} - \begin{pmatrix} -A^TY \\ \vdots \\ AX \end{pmatrix} = \begin{pmatrix} C^T \\ \vdots \\ -b \end{pmatrix}$$

$$\begin{pmatrix} u \\ \vdots \\ v \end{pmatrix} - \begin{pmatrix} 0X - A^TY \\ \vdots \\ AX - 0Y \end{pmatrix} = \begin{pmatrix} C^T \\ \vdots \\ -b \end{pmatrix}$$

$$\begin{pmatrix} u \\ \vdots \\ v \end{pmatrix} \geq 0, \begin{pmatrix} X \\ \vdots \\ Y \end{pmatrix} \geq 0 \; and \; \begin{pmatrix} u \\ \vdots \\ v \end{pmatrix}^T \begin{pmatrix} X \\ \vdots \\ Y \end{pmatrix} = 0$$

$$\begin{pmatrix} u \\ \vdots \\ v \end{pmatrix} - \begin{pmatrix} 0 - A^TY \\ \vdots \\ AX - 0 \end{pmatrix} = \begin{pmatrix} C^T \\ \vdots \\ -b \end{pmatrix} \quad \rightarrow \quad (8)$$

Conversely, $\begin{pmatrix} u \\ \vdots \\ v \end{pmatrix}^T \begin{pmatrix} X \\ \vdots \\ Y \end{pmatrix} = 0$ together satisfy all the conditions of (8) then X is a superior way to solve the linear programming problems(8). Now, considering all the vectors and matrices in (8) which are written in partitioned form as follows:

$$w = \begin{pmatrix} u \\ \vdots \\ v \end{pmatrix}, z = \begin{pmatrix} X \\ \vdots \\ Y \end{pmatrix}, M = \begin{pmatrix} 0 - A^T \\ \vdots \\ A - 0 \end{pmatrix}, \quad q = \begin{pmatrix} C^T \\ \vdots \\ -b \end{pmatrix} \quad \rightarrow (9)$$

Then, (8) is seen to be the LCP. We shall determine the complementarity feasible solution to the linear complementarity problem, which yields the Linear Programming Problem (4) results.

**Quadratic Programming Problem as a Linear complementarity problem**

Given the quadratic programming problem (QPP).
$Minimize\ CX + \frac{1}{2} X^TDX$
Subject to $AX \geq b$ → (10)
$x \geq 0$
Where A is a matrix of order $m \times n$ and D is symmetric matrix of order m. The dual of this problem is given by
$Maximize\ b^TY$
Subject to $A^TY \leq (C + X^TD)^T$ → (11)
$Y \geq 0$
Introducing the slack variable $U \in \mathbb{R}^n$, the constraint equation in (11) becomes
$A^TY + U \leq (C + X^TD)^T$
By the symmetric property of D we have
$U + A^TY \leq C + X^TD$
Thus, $U - DX + A^TY \leq C^T$ → (12)
Similarly, introducing the dual surplus variable $V \in \mathbb{R}^m$, the constraint equations in (13) becomes.
$V - AX = -b$ → (13)
Equation (12) and (13) combined together in matrix notation gives the following matrix equation.

$$\begin{pmatrix} U \\ V \end{pmatrix} - \begin{pmatrix} DX - A^TY \\ AX + 0Y \end{pmatrix} = \begin{pmatrix} C^T \\ -b \end{pmatrix} \rightarrow (14)$$

A point $X \in \mathbb{R}^n$ is considered a Kuhn Tucker point for the quadratic programming problem (10) if there exist a dual vector $Y \in \mathbb{R}^n$ and surplus vector $V \in \mathbb{R}^n$ such that X,Y,U,V together satisfy

$\begin{pmatrix} U \\ V \end{pmatrix} - \begin{pmatrix} D & -A^T \\ A & 0 \end{pmatrix}\begin{pmatrix} X \\ Y \end{pmatrix} = \begin{pmatrix} C^T \\ -b \end{pmatrix}$,

$\begin{pmatrix} U \\ V \end{pmatrix} \geq 0, \begin{pmatrix} X \\ Y \end{pmatrix} \geq 0 \; and \; \begin{pmatrix} U \\ V \end{pmatrix}^T = 0$

If we take, $W = \begin{pmatrix} U \\ V \end{pmatrix}, Z = \begin{pmatrix} X \\ Y \end{pmatrix}, M = \begin{pmatrix} D & -A^T \\ A & 0 \end{pmatrix}$ and $q = \begin{pmatrix} C^T \\ -b \end{pmatrix}$

Then (12) is seen to be a linear complementarity problem. Solving this LCP (14) provides a Kuhn Tucker point for the QPP (10). If D is a positive semi definite matrix, (that is $X^T DX \geq 0, X \in \mathbb{R}^n$) this Kuhn tucker point is an optimal solution for (10).

**Fuzzy Quadratic Programming Problem (FQPP) as a Fuzzy Linear Complementarity Problem (FLCP)**

Given the Quadratic Programming Problem given below.

Minimize $\tilde{f}(\tilde{x}) = \tilde{c}^t\tilde{x} + \frac{1}{2}\tilde{x}^t\tilde{H}\tilde{x}$

Subject to $\tilde{A}\tilde{x} \leq \tilde{b}$ and $\tilde{x} \geq 0$

In this case , $\tilde{b}$ is an m -vector and $\tilde{c}$ is an n -vector of fuzzy integers. A and H are two distinct fuzzy symmetric matrices, A being $m \times n$ and H being $n \times n$. The statement that follows is an expression for the Kuhn-Tucker conditions, let $\tilde{y}$ be the vector of slack variables, and let $\tilde{u}, \tilde{v}$ be the Lagrangian multiplier vectors of the constraints $\tilde{A}\tilde{x} \leq \tilde{b}$ and $\tilde{x} \geq 0$, respectively.

$\tilde{A}\tilde{x} + \tilde{y} = \tilde{b}$

$-\tilde{H}\tilde{x} - \tilde{A}^t\tilde{u} + \tilde{v} = \tilde{c}$

$\tilde{x}^t\tilde{v} = 0, \tilde{u}^t\tilde{y} = 0$ And $\tilde{x}, \tilde{y}, \tilde{u}, \tilde{v} \geq 0$

Now letting $\tilde{M} = \begin{bmatrix} \tilde{0} & -\tilde{A} \\ \tilde{A}^t & \tilde{H} \end{bmatrix}, \tilde{q} = \begin{bmatrix} \tilde{b} \\ \tilde{c} \end{bmatrix}, \tilde{w} = \begin{bmatrix} \tilde{u} \\ \tilde{v} \end{bmatrix} and \; \tilde{z} = \begin{bmatrix} \tilde{x} \\ \tilde{y} \end{bmatrix}$ the Kuhn-Tucker conditions are stated as the LCP.

$\tilde{W} - \tilde{M}\tilde{Z} = \tilde{q}$

$\tilde{W}^t\tilde{Z} = 0$

$(\tilde{W}, \tilde{Z}) \geq 0$

*4.2) Proposed Method NFLPP as a NFLCP*

**Step 1:** Consider the Neutrosophic Fuzzy Linear Programming Problem (NFLPP).

**Step 2:** By introducing slack dual variable $\tilde{u} \epsilon \mathbb{R}^m$ and the primal surplus variable $\tilde{v} \epsilon \mathbb{R}^n$

**Step 3:** Combine them together in matrix notation.

$$\begin{pmatrix} \tilde{u} \\ \vdots \\ \tilde{v} \end{pmatrix} - \begin{pmatrix} 0 - \tilde{A}^T\tilde{Y} \\ \vdots \\ \tilde{A}\tilde{X} - 0 \end{pmatrix} = \begin{pmatrix} \tilde{C}^T \\ \vdots \\ -\tilde{b} \end{pmatrix}$$

**Step 4:** Considering that all the vectors and matrices are written in partitioned form as follows:

$$\tilde{w} = \begin{pmatrix} \tilde{u} \\ \vdots \\ \tilde{v} \end{pmatrix}, \tilde{z} = \begin{pmatrix} \tilde{X} \\ \vdots \\ \tilde{Y} \end{pmatrix}, \tilde{M} = \begin{pmatrix} 0 - \tilde{A}^T \\ \vdots \\ \tilde{A} - 0 \end{pmatrix}, q = \begin{pmatrix} \tilde{C}^T \\ \vdots \\ -\tilde{b} \end{pmatrix}$$

**Step 5:** Apply the Neutrosophic Linear Complementarity Algorithm, we get the obtained solution.

**Proposed Method NFQPP as a NFLCP**

**Step 1:** Consider the Neutrosophic Fuzzy Quadratic Programming Problem (NFQPP)

**Step 2:** By Introducing the slack Primal variable $\tilde{U} \in \mathbb{R}^n$ and the dual surplus variable $\tilde{V} \in \mathbb{R}^m$

**Step 3:** Combine the constraints and formulate the matrix form.

$$\begin{pmatrix} \tilde{U} \\ \tilde{V} \end{pmatrix} - \begin{pmatrix} \tilde{D}\tilde{X} - \tilde{A}^T\tilde{Y} \\ \tilde{A}\tilde{X} + 0\tilde{Y} \end{pmatrix} = \begin{pmatrix} \tilde{C}^T \\ -\tilde{b} \end{pmatrix}$$

**Step 4:** Considering that all the vectors and matrices are written in partitioned form as follows:

If we take, $\tilde{W} = \begin{pmatrix} \tilde{U} \\ \tilde{V} \end{pmatrix}, \tilde{Z} = \begin{pmatrix} \tilde{X} \\ \tilde{Y} \end{pmatrix}, \tilde{M} = \begin{pmatrix} \tilde{D} & -\tilde{A}^T \\ \tilde{A} & 0 \end{pmatrix}$ and $\tilde{q} = \begin{pmatrix} \tilde{C}^T \\ -\tilde{b} \end{pmatrix}$

**Step 5:** Use the Kuhn-Tucker conditions to solve the Neutrosophic Linear Complementarity Problem,

**Step 6:** Proceed through the steps again until the complementarity feasible solution to the Neutrosophic Linear Complementarity Problem is satisfied.

**Applications to the proposed Methods**

**Numerical Example**

The Tummy-Tummy Instant Noodles manufactures products two species; vegetable specie and chicken flavor respectively, For the sale of a pack of Tummy-Tummy Instant Noodles, the profits gained per unit are \$2 and \$1, respectively. Each product is processed on two machines. The vegetable specie requires two minutes of processing time in machine one and three minutes in machine two, while the chicken flavor requires one minute of processing time in machine one and two minutes in machine two. Machine one is available for not more than four hours while machine two is available for not more than six hours during any working day. Determine the number of units of the vegetable specie and chicken flavor that can be manufactured to get minimum profit.

Solution : By formulating the LPP , we have

$Minimize\ \tilde{z} = \tilde{2}\tilde{x}_1 + \tilde{x}_2$

Subject to constraint $\tilde{2}\tilde{x}_1 + \tilde{x}_2 \geq \tilde{4}$ ,

$\tilde{3}\tilde{x}_1 + \tilde{2}\tilde{x}_2 \geq \tilde{6}$ and $\tilde{x}_1, \tilde{x}_2 \geq \tilde{0}$

Where $\tilde{A} = \begin{bmatrix} \tilde{2} & \tilde{1} \\ \tilde{3} & \tilde{2} \end{bmatrix}, \tilde{b} = \begin{bmatrix} \tilde{4} \\ \tilde{6} \end{bmatrix}, \tilde{c} = [\tilde{2} \quad \tilde{1}]$

$$\widetilde{M} = \begin{pmatrix} 0 & -\tilde{A}^T \\ \tilde{A} & 0 \end{pmatrix} = \begin{pmatrix} \tilde{0} & \tilde{0} & -\tilde{2} & -\tilde{3} \\ \tilde{0} & \tilde{0} & -\tilde{1} & -\tilde{2} \\ \tilde{2} & \tilde{1} & \tilde{0} & \tilde{0} \\ \tilde{3} & \tilde{2} & \tilde{0} & \tilde{0} \end{pmatrix}$$

$$\tilde{q} = \begin{pmatrix} \tilde{C}^T \\ -\tilde{b} \end{pmatrix} = \begin{pmatrix} \tilde{2} \\ \tilde{1} \\ -\tilde{4} \\ -\tilde{6} \end{pmatrix}$$

$$\widetilde{W} = \begin{pmatrix} \widetilde{U} \\ \widetilde{V} \end{pmatrix} = \begin{pmatrix} \widetilde{U}_1 \\ \widetilde{U}_2 \\ \widetilde{V}_1 \\ \widetilde{V}_2 \end{pmatrix} = \begin{pmatrix} \widetilde{W}_1 \\ \widetilde{W}_2 \\ \widetilde{W}_3 \\ \widetilde{W}_4 \end{pmatrix}, \qquad \tilde{Z} = \begin{pmatrix} \tilde{X} \\ \tilde{Y} \end{pmatrix} = \begin{pmatrix} \tilde{x}_1 \\ \tilde{x}_2 \\ \tilde{y}_1 \\ \tilde{y}_2 \end{pmatrix} = \begin{pmatrix} \tilde{z}_1 \\ \tilde{z}_2 \\ \tilde{z}_3 \\ \tilde{z}_4 \end{pmatrix}$$

Therefore, $\tilde{x}_1 = \tilde{z}_1$ and $\tilde{x}_2 = \tilde{z}_2$ will be an optimal solution to the LPP. $\widetilde{W} - \widetilde{M}\tilde{Z} = \tilde{q}$ gives

$$\begin{pmatrix} \widetilde{W}_1 \\ \widetilde{W}_2 \\ \widetilde{W}_3 \\ \widetilde{W}_4 \end{pmatrix} - \begin{pmatrix} \tilde{0} & \tilde{0} & -\tilde{2} & -\tilde{3} \\ \tilde{0} & \tilde{0} & -\tilde{1} & -\tilde{2} \\ \tilde{2} & \tilde{1} & \tilde{0} & \tilde{0} \\ \tilde{3} & \tilde{2} & \tilde{0} & \tilde{0} \end{pmatrix} \begin{pmatrix} \tilde{z}_1 \\ \tilde{z}_2 \\ \tilde{z}_3 \\ \tilde{z}_4 \end{pmatrix} = \begin{pmatrix} \tilde{2} \\ \tilde{1} \\ -\tilde{4} \\ -\tilde{6} \end{pmatrix}$$

which the single valued Neutrosophic coefficients are assumed to be

$\tilde{1} = \langle(0,1,2); 0.6,0.4,0.2\rangle$ ,

$\tilde{2} = \langle(1,2,3); 0.4,0.2,0.2\rangle$ ,

$\tilde{3} = \langle(2,3,4); 0.5,0.3,0.3\rangle$ ,

$\tilde{4} = \langle(2,4,6); 0.6,0.4,0.4\rangle$ and

$\tilde{6} = \langle(5,6,7); 0.4,0.3,0.2\rangle$

$-\tilde{1} = \langle(-2,-1,0); 0.6,0.4,0.2\rangle$ ,

$-\tilde{2} = \langle(-3,-2,-1); 0.4,0.2,0.3\rangle$

$-\tilde{3} = \langle(-4,-3,-2); 0.5,0.3,0.3\rangle$ ,

$-\tilde{4} = \langle(-6,-4,-2); 0.6,0.4,0.4\rangle$ and

$-\tilde{6} = \langle(-7,-6,-5); 0.4,0.3,0.2\rangle$

Solving this problem using the Lemke's complementarity pivoting algorithm, we obtain the complementarity basic feasible solution. The proposed technique 4.2 algorithm solves the neutrosophic linear complementary Problems and tabulates the outcomes. The linear complementarity problem has an optimal solution in $\tilde{X} = (\tilde{x}_1, \tilde{x}_2) = (\tilde{z}_1, \tilde{z}_2) = \left(\left\langle \begin{matrix} \mathbf{(0.6,2,6)}; \\ \mathbf{0.6,0.2,0.2} \end{matrix} \right\rangle, \left\langle \begin{matrix} (0,0,0); \\ 1,0,0 \end{matrix} \right\rangle\right) = (2,0)$ The conclusion to this problem, $\tilde{x}_1 = 2, \tilde{x}_2 = 0$, occurs exactly when the linear program's simplex algorithm is executed.

**Numerical Example**

The LG Ltd, has two production sites, one located at Thanjavur District and the other at Mayiladuthurai District. Each site produces three items Televisions, Air condition and Projectors. The production number of each item in thousands is produced per day are as follows: A market survey indicate that during the month of May there will be a demand of 4,000 Televisions, 2000 Air conditions and 4,000 Projectors. The operating costs per day in hundreds for items in Thanjavur District and Mayiladuthurai District are 2 and 3 respectively take away 2 times the square of the quantity produced in Thanjavur District. For how many days each item be run in May so as to maximize the production cost?

**Solution :**

We formulate the fuzzy quadratic programming problem

$$Maximize\ \tilde{z} = \tilde{2}\tilde{x}_1 + \tilde{3}\tilde{x}_2 - \tilde{2}\tilde{x}_1{}^2$$

Subject to constraint $\tilde{x}_1 + \tilde{4}\tilde{x}_2 \leq \tilde{4}$ , $\tilde{x}_1 + \tilde{x}_2 \leq \tilde{2}$ , $\tilde{x}_1 + \tilde{x}_2 \geq \tilde{4}$ and $\tilde{x}_1, \tilde{x}_2 \geq \tilde{0}$

Converting this problem to minimization problem, we have

$$Minimize\ \tilde{z} = -\tilde{2}\tilde{x}_1 - \tilde{3}\tilde{x}_2 + \tilde{2}\tilde{x}_1{}^2$$

Subject to constraint $-\tilde{x}_1 - \tilde{4}\tilde{x}_2 \geq -\tilde{4}$ , $-\tilde{x}_1 - \tilde{x}_2 \geq -\tilde{2}$ , and $\tilde{x}_1, \tilde{x}_2 \geq \tilde{0}$

If we compare this minimization problem with the QPP (10) we have

Where $\tilde{D} = \begin{bmatrix} \tilde{4} & \tilde{0} \\ \tilde{0} & \tilde{0} \end{bmatrix}, \tilde{b} = \begin{bmatrix} -\tilde{4} \\ -\tilde{2} \end{bmatrix}, \tilde{c} = [-\tilde{2} \quad -\tilde{3}]$ in

Now, going by the formulation (12) of the LCP, we have the following matrices

$$\tilde{M} = \begin{pmatrix} \tilde{D} & -\tilde{A}^T \\ \tilde{A} & 0 \end{pmatrix} = \begin{pmatrix} \tilde{4} & \tilde{0} & \tilde{1} & \tilde{1} \\ \tilde{0} & \tilde{0} & \tilde{4} & \tilde{1} \\ -\tilde{1} & -\tilde{4} & \tilde{0} & \tilde{0} \\ -\tilde{1} & -\tilde{1} & \tilde{0} & \tilde{0} \end{pmatrix}$$

Solving the LCP $(\tilde{M}, \tilde{q})$ will result to solving the quadratic programming problem. That is, the values of $\tilde{z}_1$ and $\tilde{z}_2$ obtained from the LCP $(\tilde{M}, \tilde{q})$ is the solution to the QPP.

$$\tilde{q} = \begin{pmatrix} \tilde{C}^T \\ -\tilde{b} \end{pmatrix} = \begin{pmatrix} -\tilde{2} \\ -\tilde{3} \\ \tilde{4} \\ \tilde{2} \end{pmatrix}$$

which the single valued Neutrosophic coefficients are assumed to be

$\tilde{1} = \langle(0,1,2); 0.2,0.3,0.5\rangle$ ,

$\tilde{2} = \langle(0,2,4); 0.6,0.4,0.1\rangle$ ,

$\tilde{2} = \langle(1,2,3); 0.2,0.3,0.5\rangle$ ,

$\tilde{3} = \langle(2,3,4); 0.6,0.4,0.1\rangle$

$\tilde{4} = \langle(0,4,8); 0.4,0.3,0.2\rangle$

and $\tilde{4} = \langle(2,4,6); 0.6,0.4,0.1\rangle$

Using Lemke's complementarity pivoting algorithm the LCP formulated above yields the complementarity basic feasible solution. The neutrosophic linear complementary problem has been solved by the suggested method 4.3 algorithm, and the resulting findings are tabulated.

Therefore, the crisp value resulting from solving this linear complementarity problem is $(W, Z) = (w_1, w_2, w_3, w_4, z_1, z_2, z_3, z_4) = \left(0,0,0,\frac{49}{64},\frac{5}{16},\frac{59}{64},\frac{3}{4},0\right)$

## CONCLUSION

This study proposes an innovative method to solve neutrosophic fuzzy quadratic programming problems and neutrosophic fuzzy linear programming problems as fuzzy linear complementarity Problem with neutrosophic fuzzy parameters. Here, the Linear Complementarity Problem with Single Valued Triangular Neutrosophic Numbers (SVTNN) has been resolved with the Principle of Pivoting technique. Real-world examples also strengthen the proposed method.

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**Table 01. Production sites**

| Number of items | Thanjavur District | Mayiladuthurai District |
|---|---|---|
| Televisions (T) in 1000 | 1 | 4 |
| Air Conditions (A) in 1000 | 1 | 1 |
| Projectors (P) in 1000 | 1 | 1 |

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**RESEARCH ARTICLE**

# Nutritional Factors in the Prevention and Management of Chronic Kidney Disease

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## ABSTRACT

One of the primary strategies for boosting health and preventing diseases of all types, including renal ailments, is nutrition. Chronic kidney disease, which affects numerous metabolic pathways and is often progressive and irreversible, is defined as evidence of structural or functional renal impairment for three months or more. Altered protein homeostasis, hormonal dysfunction, aberrant protein catabolism, and acid-base disturbances ensue. Diet is one of the biggest modifiable risk factors for chronic kidney disease (CKD)-related death and disability. Chronic kidney disease (CKD) is a major public health concern that requires comprehensive management for preventing and delaying the progression to advanced CKD. This review examines the most recent research on the processes underlying diet-induced kidney disease with a focus on the role of diet in the development and progression of CKD. It is possible that improving diet quality or limiting dietary intake could be used as an adjunct therapy for CKD prevention or progression in susceptible individuals, thereby reducing the burden of CKD. We gathered this data from published researchers from PubMed, Science Direct, Google open access publications, Excerpta Medica Database, web of science from the year 2016-2023. Since more than a century ago, nutritional therapy has been advocated as a way to decrease the progression of CKD and uremia. Historically, the management of CKD has relied on dietary therapy, such as sodium and protein restriction. Other nutrients may affect kidney function in a number of ways, including through the change of gut homeostasis, metabolic or hemodynamic pathways, or all three. A diet rich in fruits and vegetables during the early stages of CKD may decrease the disease's progression, according to growing evidence; however, more research is required before guidelines are changed. For those who frequently drink sugar-sweetened beverages and eat a lot of fructose through their diet, reducing intake may be beneficial.

Additional high-quality research is required to fully determine the impact of these dietary factors in CKD patients. More study is needed to develop a more thorough, evidence-based approach to the nutritional management of chronic kidney disease.

**Keywords:** CDK (chronic kidney disease); Diet; Nutritional therapy; Homeostasis; Allopathic drugs.

## INTRODUCTION

Chronic kidney disease, which affects numerous metabolic pathways and is often progressive and irreversible, is defined as evidence of structural or functional renal impairment for three months or more.Using the KDOQI [1,2] standards, CKD can be divided into five stages utilising thresholds of eGFR within the CKD range and/or evidence of structural renal abnormalities, such as proteinuria. Stage 3 should be separated into 3a and 3b to represent the rising CVD risk, according to NICE [3]. A UK retrospective lab audit research found that CKD stage 3 was the most prevalent stage, accounting for nearly 90% of cases, with 84% stage 3a (GFR of 45 to 59 ml/min/173m2) and 16% stage 3b (GFR of 30 to 44 ml/min/173m2 [4]. Although CKD can develop in the absence of disease owing to ageing, exposure to chemicals, or infection, it can also happen as a result of chronic disorders including diabetes, obesity, hypertension, or cardiovascular disease , addressing the underlying causes of CKD (diabetes and hypertension), obtaining and maintaining appropriate nutritional status and nitrogen balance, and avoiding are all goals of nutrition interventions in CKD [1]. Further moving to symptoms of CKD it includes the development of anaemia, cognitive changes, hypertension, gastrointestinal problems, shortness of breath, kidney alterations, changes in output, and anaemia, itch and cramps, glomerular capillary wall and tube damage, as well as peripheral oedema brought on by salt. While comparing all anemia, bone disorders, CVD, and cancer diseases are the most common CKD consequences [5].Now coming to management the current recommendations of Kidney Outcomes Quality Initiative (KDOQI) and National Institute for Health Excellence (NICE) are to utilise serum creatinine concentration to estimate GFR (eGFR) and transform it using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [2,3]. The Modification of Diet in Renal Disease (MDRD) equation is replaced by the CKD-EPI equation as a more reliable predictor of clinical risk, and both of these models account for specific non-renal effects (age, race, gender)[6,7]. Dietary habits and nutrition are linked to the onset of chronic metabolic illnesses, and they can be changed in order to stop or reduce the progression of CKD [8]. Following recommended dietary guidelines can lower the risk of developing CKD or halt its progression, and enhance overall health and death [9].The possibility that many other substances may affect kidney health through metabolic or hemodynamic effects, modulation of gut homeostasis, including changes to gut microbiota, or other mechanisms is becoming more and more supported by research. The goal of this review is to provide an overview of recent literature on potential novel modifiable dietary components that may be helpful in the treatment of CKD and to summarise the most important suggestions made to date.

**Main Text**

Chronic kidney disease, which affects numerous metabolic pathways and is often progressive and irreversible, is defined as evidence of structural or functional renal impairment for three months or more[10].Hormonal dysfunction, abnormal protein catabolism, altered protein homeostasis, and acid-base disturbances ensue[11].However, as chronic kidney disease progresses, the accumulation of nitrogen-containing byproducts from dietary and intrinsic protein catabolism may distort taste and smell and reduce appetite[11]. Since uremia affects the microbiome and disturbs intestinal epithelia, gastrointestinal nutrition absorption ultimately becomes aberrant[12].As a result, nutritional status often becomes disordered, and protein energy wasting is common, requiring dietary adjustments in this population .Nutritional treatment, however, may support the management of uremia in addition to dietary modifications, as well as other issues such electrolyte and acid-base imbalances, water and salt retention, mineral and bone abnormalities, and failure to thrive. In fact, depending on the patient's preferences, dietary treatments may also be employed to delay or prevent dialysis therapy or for the conservative

management of uremia. The diet of CKD patients should be a key factor in their care since nutritional therapy of individuals with CKD is thought to decrease uremic symptoms and provide positive effects on the progression of renal dysfunction. Numerous recommendations urge these people to consume less protein because a high-protein diet makes kidney disease worse. Limiting salt intake is often advised since excessive sodium may be linked to the progression of CKD and all-cause mortality. High potassium is linked to heart arrhythmia, while low potassium is linked to muscle weakness and hypertension. In order to keep serum potassium levels within the normal range, new guidelines advise modifying dietary potassium consumption on an individual basis. For individuals with CKD G3, G4, an appropriate dietary calcium intake is advised to maintain calcium balance. For patients with CKD, individualized approaches are required to ensure the best outcome.

**Major Role of Kidney in Metabolic Regulation**

**Water Electrolyte Haemostasis**

The objective of the study was to evaluate the physiological effects of routine water consumption to those of electrolyte drinks in terms of exercise capability and recovery following strenuous activity. Ten healthy young men took part, working out on a treadmill before and after getting regular water and an electrolyte drink (3RINK) four weeks later. 30 minutes before activity and just after it, 250 mL of fluid were consumed.Haematocrit values indicated a fluid type and time period connection. Between P0 and P1, there were noticeably varied sodium concentrations in the various fluid types. Ultimately, a relationship between each type of fluid and serum lactate levels was discovered. Hence, consuming 3RINK before and after strenuous activity improved exercise performance, decreased weariness from exercise, and boosted the body's capacity to retain water[13].

**Calcium Phosphate Balance.**

The amount of a substance created (or absorbed from the intestine) must match the amount expelled in order to maintain homeostasis when GFR decreases. Each undamaged nephron excretes a higher percentage of its filtered load as nephron loss progresses. For instance, whereas at normal GFR levels each nephron may only need to excrete 10% of its filtered phosphate load to maintain homeostasis, 90% may need to be expelled by leftover nephrons. Despite these adjustments, when CKD worsens, the remaining nephrons' capacity to excrete the daily load of phosphate eventually declines, leading to a positive balance[14].

**Waste Product Removal**

Through minuscule filtering organs known as nephrons, the kidneys remove waste products from the blood known as urea. Each kidney contains a million or so nephrons. The glomerulus is where blood is filtered after entering. This filtered fluid then travels through the tubule where materials and water are added to or subtracted from the mixture. Urine is the remaining liquid. Following formation, urine travels down the renal tubules of the kidney before passing through the nephrons. The calyces serve as a collection point for urine, which then flows into the renal pelvis and into the ureter. From there, it descends and enters the bladder[15].

**Acid base Balance**

For the most part, the kidneys rely on 2 main mechanisms to keep the acid-base balance. Reabsorption of the filtered bicarbonate is the first step. A healthy glomerulus can filter 4,500 mEq of bicarbonate per day, and the proximal tubule can reabsorb up to 80% of the filtered bicarbonate [16]. Metabolic acidosis, hypokalemia, hypovolemia, angiotensin II, and glucocorticoids are some of the conditions that can increase proximal tubule reabsorption of bicarbonate. The second procedure entails the production of fresh bicarbonate through the release of distal nephron acid and ammonia. Even the most acidified urine cannot excrete the daily acid load in the absence of buffering, so the H+ ions secreted into the urine are largely bound by H+ acceptors. This function is carried out by buffers like urine phosphate, which are frequently referred to as titratable acids. However, the kidney's production of ammonia, which multiplies several-fold more than titratable acid excretion, is the main mechanism of new bicarbonate generation in the context of metabolic acidosis. Ammonia and bicarbonate are produced in the proximal tubule as a result of the metabolism of glutamine to glutamate and then oxaloacetate. There may also be a minor role for other metabolic pathways [17]. If the kidney's ammonia is released back into the bloodstream, the liver uses bicarbonate to process it.

As a result, the amount of base that has been added to the bicarbonate pool can be determined by the amount of ammonium that is excreted in the urine, which is necessary for net bicarbonate gain.

**Erythropoietin Production**

An essential erythropoietic hormone, erythropoietin (Epo), is primarily produced by renal Epo-producing cells (REPs). To maintain tissue oxygen homeostasis, epo production in REPs is tightly controlled in a hypoxia-inducible manner.Notably, myofibroblast-transformed REPs (MF-REPs) regain their initial physiological characteristics after the disease insults have been resolved, indicating that renal anaemia and fibrosis may be partially reversible [18].

**Degradation of Peptides**

Despite receiving an adequate diet, people with chronic kidney disease (CKD) and other catabolic diseases (such as sepsis, diabetes, and cancer) can still experience muscle wastin Due to the distinctive 14kD actin fragment that caspase-3 activity leaves in muscle samples, it is simple to identify caspase-3 activity. The degree of muscle degradation in dialysis patients and in other catabolic conditions correlates well with this fragment, according to preliminary data from studies in patients and a number of experimental models of catabolic diseases [19].

**Blood Pressure Regulation**

Patients with chronic kidney disease (CKD) who have uncontrolled blood pressure (BP) run the risk of serious negative effects. Achieving a healthy blood pressure level is crucial for preventing cardiovascular events (CVEs) and end-stage kidney disease. Notably, the risk of poor kidney outcomes is elevated with this lower target SBP. Unfortunately, there haven't been any studies on this topic that have only included people with CKD, especially those with advanced CKD[20].

**Symptoms**

Many patients with CKD are asymptomatic and don't exhibit any symptoms until they are sick with severe CKD oruntil they get a chance finding from screening testing, such as during a routine physical or checkup. However, depending on the underlying cause ofCKD, some individuals have symptoms as a direct result of their deteriorated kidney health

- Epistaxis
- Pigmentation
- Anorexia Nausea
- Vomiting
- Diarrhoea
- Coma
- Fits (severe uraemia)
- Uraemic pericarditis
- Hypertension
- Peripheral vascular disease
- Heart failure
- Nocturia
- Polyuria
- Bone pain
- Hyperparathyroidism
- Osteosclerosis

**Diagnosis**

❖ **Blood tests:** During kidney function tests, the presence of waste products in your blood, such as urea and creatinine, is measured.

- **Urine tests:** A urine sample analysis can reveal irregularities that suggest chronic kidney failure and assist in determining the underlying cause of chronic kidney disease.
- **Imaging tests:** To evaluate the structure and size of your kidneys, your doctor might use ultrasound. In some situations, different imaging tests might be used.
- **The removal of a kidney tissue sample for analysis:** A kidney biopsy, which involves taking a sample of kidney tissue, may be advised by your doctor. A long, thin needle is inserted through your skin and into your kidney during a kidney biopsy procedure under local anaesthesia. The biopsy sample is sent to a lab for analysis in order to help diagnose the kidney issue you are having.

**Causes of chronic kidney disease**

a. Diabetes type 1 or type 2
b. High blood pressure
c. Glomerulonephritis, an inflammation of the kidney's filtration cells (gloe-mer-u-low-nuh-FRY-tis) (glomeruli)
d. Other inherited kidney illnesses, such as polycystic kidney disease
e. Persistent obstruction of the urinary tract caused by diseases like an enlarged prostate, kidney stones, or certain malignancies
f. Vesicoureteral (ves-ih-koe-yoo-REE-tur-ul) (ves-ih-koe-yoo-REE-tur-ul) reflux, a medical disorder when urine backs up into the kidneys
g. Pyelonephritis, another name for recurrent kidney infection (pie-uh-low-nuh-FRY-tis)

**Risk factors**

- Obesity
- Diabetes
- Older age
- High blood pressure
- Smoking
- Heart (cardiovascular) disease

**Complications of chronic kidney disease:**

- Anemia
- Gout
- Fluid retention
- High blood pressure (hypertension)

**Long-term complications:**

- Kidney failure
- Heart disease
- Weakened immune system

**Pathophysiology**

Renal insufficiency or reduced renal reserve are the earliest symptoms of chronic kidney disease (CKD), which can eventually lead to renal failure (end-stage renal disease). There are initially few obvious anomalies as renal tissue begins to function less because the remaining tissue performs better. The ability of the kidneys to maintain fluid and electrolyte homeostasis is hampered by decreased renal function. Early falls in urine concentration are followed by declines in urine excretion of excess phosphate, acid, and potassium. The ability to efficiently dilute or concentrate urine is lost when renal failure is advanced (glomerular filtration rate [GFR] 15 mL/min/1.73 m2); as a result, urine osmolality is typically fixed at between 300 and 320 mOsm/kg, which is comparable to plasma (275 to 295 mOsm/kg).

**Nutritional management:**
**Role of Dietary Constituents in Kidney Disease**
**Protein:**
**Protein amount**
One of the most frequently addressed topics in the dietary management of CKD is protein intake. It has been demonstrated that a high-protein diet, which is defined as consumption of more than 1.2 grammes of protein per kilogramme of body weight per day (g/kg/day), results in a considerable decline in kidney function[22]. According to animal models, a diet low in protein causes the glomerular afferent arterioles to constrict, lowering intraglomerular pressure, whereas a diet high in protein causes the afferent arterioles to dilate, increasing glomerular filtration[23]. Glomerular hyperfiltration itself has the potential to harm the remaining glomeruli over time [24]. Hence, a low-protein diet has a preglomerular effect that may improve the postglomerular action of angiotensin pathway modulators, which enlarge the efferent arterioles and subsequently reduce intraglomerular pressure[25]. For adults with moderate-to-advanced kidney disease (estimated glomerular filtration rate [eGFR], 45 ml per minute per 1.73 m2 of body surface area) and for the management of sub-substantial proteinuria (urinary protein excretion, >0.3 g per day), 0.6 to 0.8 g per kilogramme of body weight per day is the most frequently recommended target. But, the so-called extremely low-protein diet, which provides only 0.6 g of protein per kilogramme of body weight each day, is also employed [26].

Individuals with diabetes mellitus, hypertension, polycystic kidneys, or those who have undergone nephrectomy for kidney donation or cancer therapy may benefit from modest protein consumption (1 g per kilogramme per day) in order to maintain a somewhat low intraglomerular pressure[27].

**Protein type**
Depending on the source from where the protein is extracted, the protein is classified into two types i.e. Plant protein, Animal protein. Animal protein is made up of sulfur-containing amino acids that, when oxidised, produce sulphate, a non-metabolizable anion that adds to the body's overall acid burden. Higher quantities of glutamate, an anionic amino acid whose metabolism consumes hydrogen ions to remain neutral and lowers acidity levels, are found in protein from plant sources[28].According to the Chronic Renal Insufficiency Cohort Study findings, patients with CKD who consume a higher percentage of protein from plant sources have higher bicarbonate levels and better phosphorus balance[29].Although there are few human studies comparing the effects of plant-based protein to animal-based protein, what is known so far indicates that improving kidney function involves eating more plant-based protein. Proteinuria, urine creatinine, and indicators of cardiovascular disease were all improved when animal proteins were substituted with soy proteins, according to a longitudinal controlled trial in individuals with diabetic nephropathy[30]. Another study in healthy people indicated that consuming vegetable protein decreased glomerular filtration rate, enhanced albumin clearance, and was independent of total protein intake[31].A recent study compared the impact of a typical mixed-source low protein diet (0.6 g/kg/day) to a very low protein vegetarian diet (0.3 g/kg/day) on the progression of CKD. Significantly fewer individuals in the very low protein vegetarian diet group needed renal replacement therapy or had achieved the primary end-point of a GFR reduction of >50% at an 18-month follow-up[32]. Considering the available data, it is possible that individuals with CKD who consume more plant-based protein may experience better renal outcomes, given that there is enough protein to prevent protein energy loss.

**Sodium**
People who consume a high-sodium diet (>4 g of sodium per day), have underlying hypertension, or are over 55 years old are more likely to experience the association between dietary sodium intake and blood pressure [33]. Dietary sodium restriction is always advised in chronic kidney disease patients to manage fluid retention, hypertension, and to lower cardiovascular risk factors[34]. However, whether dietary sodium chloride restriction can halt the progression of kidney disease once it has already started is unclear. There aren't many pertinent interventional data for these patients because renal disease patients are frequently excluded from cardiovascular trials involving dietary sodium restriction [35]. Reduced sodium intake may help lower intraglomerular pressure by

enhancing the effects of a low-protein diet and angiotensin-modulation therapy [36]. It may also reduce proteinuria and slow the progression of kidney disease [37].

Data from observational studies using urinary sodium excretion as a proxy for sodium chloride intake have been inconsistent, with some studies finding no correlation between dietary sodium intake and the progression [38] of renal disease and others finding a positive correlation [39]. The highest quartile of urinary sodium excretion (4.5 g per day), compared to the lowest quartile (2.7 g per day), was associated with 45% higher mortality and a 54% higher risk of disease progression [39], according to a longitudinal study published in 2016 that involved serial 24-hour urine collections from 3939 patients with chronic kidney disease. When dietary sodium intake exceeded 4 g per day, adverse cardiovascular outcomes were seen [40].

Observations in the general population point to a J-shaped relationship; dietary sodium intakes of more than 5 g per day and less than 3 g per day are both linked to an increased risk of cardiovascular disease and death [41]. There is no proof that patients with kidney disease will benefit from this extremely low level of sodium restriction, even though it is frequently advised that they limit their daily dietary intake of sodium to less than 2.3 g (or 100 mmol) for those with cardiovascular disease. As a result, a daily dietary sodium intake of less than 4 g (174 mmol) is advised for the general management of chronic kidney disease and its risks, and a sodium intake of less than 3 g (131 mmol) is advised for the specific management of symptomatic fluid retention or proteinuria. Given the risk of hyponatremia and negative outcomes, there is insufficient evidence to support a sodium intake for patients with renal insufficiency of less than 1.5 g per day (87 mmol per day). Additionally, patients with certain conditions, such as salt-losing nephropathies, shouldn't be subjected to such a strict sodium restriction [42].

Patients with renal insufficiency typically experience isosthenuria, despite the fact that sufficient fluid intake may reduce the risk of kidney disease [43]. This is the rationale behind the recommendation that people with stage 3 chronic kidney disease restrict their daily fluid intake to less than 1.5 litres in order to prevent hyponatremia [42]. It is crucial to adjust this limit for hot climates and other situations with high insensible fluid losses. Given the association between symptoms of fluid retention or hyponatremia and poor outcomes in chronic kidney disease, loop diuretics are frequently prescribed as adjunctive therapy, especially for patients who have a tendency to exhibit these symptoms [42].

**Phosphorus**

Higher plasma phosphorus levels have been linked to an increased risk of incident kidney disease in the general population [44]. Given the high tissue and circulating levels of parathyroid hormone and fibroblast growth factor 23 (FGF-23) in renal insufficiency, which promote urinary phosphorus excretion, overt hyperphosphatemia is uncommon in stages 1, 2, and 3 of chronic kidney disease [45]. It is crucial to manage dietary phosphorus intake even in patients who don't appear to have hyperphosphatemia because elevated levels of parathyroid hormone and FGF-23 can lead to renal bone disease, left ventricular hypertrophy, vascular calcification and accelerated progression of kidney disease from vascular and tubulointerstitial injury [46]. The quantity and bioavailability of phosphorus vary depending on the type of protein, despite the fact that a low-protein diet also lowers phosphorus intake. As an illustration, the ratio of phosphorus to protein in egg whites and yolks, which have 3.6 and 2.7 g of protein per egg, respectively, is 1 to 2 mg and 20 to 30 mg per gram, respectively [47]. Plants (along with fibres) have a lower ability to absorb phosphorus through the digestive tract than meat (30–50% vs. 50–70%) [48], primarily in the form of phytates. Processing foods increase the phosphorus burden because they contain easily absorbed inorganic phosphorus in food additives [49]. Patients with moderate-to-advanced kidney disease should limit their dietary phosphorus intake to less than 800 mg per day (26 mmol per day), and processed foods with a high phosphorus-to-protein ratio should be avoided. An excessively strict restriction of protein intake to control hyperphosphatemia, however, may be linked to negative outcomes in patients with stage 5 chronic kidney disease who are receiving dialysis therapy or who are more at risk for protein-energy wasting [50]. Therefore, an individual dietary strategy that makes good use of phosphorus binders is preferred [51].

**Potassium**

Reducing consumption of high sodium foods typically results in an increase in the quantity of potassium in the diet, in addition to the direct advantages on blood pressure and proteinuria [52]. With their high fibre and vitamin content as well as their low acidogenicity, a lot of potassium-rich meals, like fresh fruits and vegetables, are considered as healthy options by the majority of people[53].Sodium sensitivity may be eliminated by potassium, which is known to have antihypertensive properties. The Dietary Approaches to Stop Hypertension (DASH) diet, which has twice as much potassium as a typical western diet, may be beneficial at lowering blood pressure due to an improved salt to potassium ratio[52]. Due to its high protein, potassium, calcium, and phosphorus content, the DASH diet has not been widely assessed in patients with CKD[54]. However a larger consumption of dietary potassium might be linked to a higher risk of renal disease development[55]. According to epidemiological research, both relatively low plasma potassium levels (4.0 mmol/liter) and high levels (>5.5 mmol/liter) are linked to a faster progression of renal disease [56].Impaired potassium excretion in those with severe CKD, such as those on dialysis, causes hyperkalaemia, which is associated to a higher all-cause mortality rate[57]. In individuals with extremely advanced chronic kidney disease, the highest quartile of dietary potassium consumption, compared to the lowest quartile, is associated with a 2.4-fold greater risk of death; the association is unrelated to the plasma potassium level and other dietary factors[58]. In patients with hyperkalemia, especially those with more severe stages of kidney disease, dietary potassium restriction is frequently advised. Excessive dietary limitations, on the other hand, can expose patients to less heart-healthy and more atherogenic diets[59]and worsen constipation, which may actually lead to increased intestinal potassium absorption[60]. In patients with a propensity toward hyperkalemia (>5.5 mmol of potassium per liter), a dietary potassium intake of less than 3 g per day (<77 mmol per day) is s, with the stipulation that a balanced intake of fresh fruits and vegetables with high fiber should not be compromised. In people who had decreased renal function at baseline, the DASH diet was still helpful, according to a small retrospective research[54]. Before CKD patients can be advised to follow the DASH diet, larger studies are necessary. The advantages of a plant-based diet, which are naturally high in potassium and fibre and low in acidogenic proteins and minerals, would probably outweigh the potential risk of hyperkalemia in the early stages of CKD. Treatment of metabolic acidosis with base-producing vegetables was successful in lowering renal damage and improving metabolic acidosis in a sample of stage 4 CKD patients who were chosen because they were at low risk for hyperkalaemia[61]. Larger long-term trials in CKD patients investigating plant-based diets on renal biomarkers and clinical outcomes are warranted on the basis of the positive findings to date.

**Vitamin D**

When circulation vitamin D levels in patients with chronic renal disease have been found to be low, native vitamin D supplementation (cholecalciferol or ergocalciferol) may be recommended. Vitamin D analogues have been linked in several studies to reduced proteinuria as well as the repair of renal osteodystrophy [62]. The conversion of 7-dehydrocholesterol in the skin by UV radiation into vitamin D precursors, which are supplied from the food or from the metabolism of vitamin D precursors into its active state, requires the kidneys. These precursors are transformed into calcidiol (25 hydroxy vitamin D) in the liver, which is then transformed into calcitriol (1,25-dihydroxy vitamin D3) in the mitochondria of the proximal convoluted tubules of the kidney by an enzyme known as renal 1-hydroxylase. The production of calcitriol directly decreases as kidney function deteriorates [63]. Calcitriol inhibits the release of parathyroid hormone (PTH), however in chronic kidney disease (CKD), this process is blunted by decreased calcitriol synthesis, resulting in an excessive release of parathyroid hormone, a condition known as secondary hyperparathyroidism. Renal osteodystrophy may occur as a result of this secondary hyperparathyroidism, which can also affect bone turnover and metabolism [64]. It is well known that chronic kidney disease consequences include vitamin D insufficiency and secondary hyperparathyroidism [65].

**CKD , Low Vitamin D, and Association with Mortality**

Cardiovascular disease significantly increases the risk of death in individuals with renal failure, and 20% to 30% of deaths among dialysis patients are due to sudden cardiac death[66]. The risk of sudden cardiac death is three times higher in haemodialysis patients with a severe vitamin D deficit (25 nmol/L of 25(OH)D) than it is in those with vitamin D levels greater than 75 nmol/L [67]. A meta-analysis of data from observational studies revealed that the

relative risk of death decreased significantly (RR = 0.86, CI: 0.81-0.92) for each rise in serum 25(OH)D levels of 25 nmol/L[68]. The production of extracellular insoluble calcium phosphate and consequent calcification of the vasculature can be caused by altered vitamin D levels and hyperparathyroidism[60]. Patients with CKD have been found to have coronary artery calcification[69], and calcium phosphate levels have been linked to a higher mortality risk in HD patients[70]. Increased albuminuria is also more likely to occur in those with low plasma 25(OH)D, particularly those who consume large amounts of sodium[71]. Hence, it is thought that restoring vitamin D levels may lower PTH levels, correct alterations in bone turnover and calcium metabolism, and subsequently lower mortality in the CKD population.

**Vitamin D Supplementation and Clinical Outcomes—Mortality**

While vitamin D therapy has been proven to change certain biochemical parameters in CKD patients, its impact on morbidity and mortality outcomes is less certain. A meta-analysis of observational studies revealed that individuals with CKD who used vitamin D supplements had a lower risk of cardiovascular and all-cause mortality[72]. however, a recent meta-analysis focused on RCTs that evaluated the impact of vitamin D supplementation on all-cause and cardiovascular mortality in CKD patients found no evidence that supplementation had an impact on mortality outcomes[73]. Almost two thirds of the patients in this meta-analysis had follow-up periods of less than a year, and it has been noted that this may not have been enough time to capture CVD events, which are a significant cause of mortality in the CKD group[74]. Instead of using clinical endpoints like progression to ESRD or mortality, many studies evaluating the effectiveness of vitamin D in CKD patients utilize biochemical outcomes like parathyroid levels or proteinuria[63]. In a recent comprehensive analysis, it was discovered that there was insufficient proof in favour of vitamin D supplementation for a number of health outcomes, including chronic kidney disease[75]. Those with early chronic renal disease and secondary hyperparathyroidism should take vitamin D supplements, according to current Australian guidelines, but they acknowledge that there is no proof that doing so improves patient-level outcomes[65]. So, despite the fact that vitamin D may significantly change biochemical parameters, larger, longer randomised control studies are urgently needed to determine whether these changes correspond to significant patient-centered outcomes.

**Calcium**

A positive calcium balance may result from passive diffusion of ionised calcium, which is exacerbated by decreased urinary calcium excretion brought on by secondary hyperparathyroidism. The decline in 1,25-dihydroxyvitamin D associated with renal insufficiency reduces gastrointestinal absorption of calcium[76]. In hyperactive renal bone disease, greater calcium release from bone (increased bone resorption due to secondary hyperparathyroidism) improves the positive calcium balance and may worsen vascular calcification[77]. Differences in dissociation and bioavailability between different types of elemental calcium induce variations in gut calcium absorption; for example, calcium citrate is more easily absorbed than calcium acetate[76]. According to two studies, individuals with stage 3 or stage 4 chronic renal disease may be able to maintain a table calcium balance by consuming 800 to 1000 mg of elemental calcium daily (20 to 25 mmol daily)[76,78]. As a result, patients with moderate-to-advanced chronic renal disease only need 800 to 1000 mg of elemental calcium per day from all sources, as compared to the 1000 to 1300 mg (25 to 32 mmol)[76]recommended for people without kidney disease[76,78].

**Carbohydrates**

Unrefined carbs make over half of the average daily energy consumption, and a low-protein diet may increase this percentage even further. In patients with renal disease should consume complex carbohydrates with a high fibre content, such as whole-wheat breads, multigrain cereal, oatmeal, and mixed fruits and vegetables, to assist lower the production of urea and creatinine as well as dietary phosphorus and protein[79].To avoid weight gain on a low-protein diet, fat and carbohydrates should account for more than 90% of the daily energy requirement of 30 to 35 kcal per kilogramme [80].The maintenance of correct glycemic control in patients with diabetic kidney disease is obvious, but adequate energy intake is necessary to reduce the danger of protein-energy wasting and hypoglycemia, which rises as kidney function deteriorates.

**Dietary patterns**

**Mediterranean Diet**

The United Nations Educational, Scientific and Cultural Organization (UNESCO) officially recognised MD use as "an intangible cultural legacy of humanity" in 2010 for its contribution to maintaining individual health and having a favourable impact on longevity [84].Regular use of plant-based foods such fruit, vegetables, legumes, grains, and nuts, as well as the usage of EVOO, are characteristics of the MD. Also, it is renowned for being the primary source of vegetable lipids and for permitting the occasional glass of red wine[85,86].The MD is rich in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) due to the use of EVOO and nuts, while yet maintaining a low intake of saturated fatty acids (SFAs), which come from foods like dairy and meat[87].Also, the MD is abundant in foods that are strong in antioxidant and anti-inflammatory substances, which can fend off a number of disorders linked to chronic inflammation and oxidative stress. Among these foods, EVOO (which is high in polyphenols and MPCs) has a number of beneficial effects on CKD patients, including reducing inflammation and oxidative stress while enhancing purine and lipid metabolism[88,89].In conclusion, treating metabolic acidosis in the early stages of CKD with the MD can be a beneficial nutritional dietary strategy. To prevent hyperkalemia in the later phases, the potassium intake must be under control. In fact, dietary therapy using LPD or vegan LPD appear to be more successful in moderate-to-severe CKD[22].

**Fruits and vegetables**

In order to minimise dietary acid load by 50%, a diet high in fruits and vegetables was given. The potential renal acid load, 8-hour urine net acid excretion, urine albumin-to-creatinine ratio, and urine TGF- significantly improved in patients with stage 2 CKD who underwent this dietary intervention for 30 days. Chronic kidney disease (CKD), estimated glomerular filtration rate (eGFR), and transforming growth factor (TGF-) are acronyms[90].In this regard, it is important to teach patients how to boil their vegetables, either twice or for a long period of time, before eating them. Fruit consumption should also increase. Low-potassium fruit (such as apples, pears, strawberries, etc.) should only be consumed in amounts of no more than 300 g per day. Fruit heavy in potassium, such bananas, peaches, etc., should be avoided[91].

**DASH Diet (Dietary approaches to stop hypertension)**

Legumes, whole grains, vegetables, fruits, low-fat dairy products, moderate amounts of chicken, fish, and nuts, as well as small amounts of sodium, red/processed meats, alcohol, and sweetened beverages, are all included in the DASH diet, which is a comprehensive eating pattern [92]. The DASH diet was studied in three studies: the ARIC research with 23 years of follow-up, the Iranian TLGS with 6.1 years of follow-up, and the Healthy Aging in Neighborhoods of Diversity throughout the Life Span cohort with 5 years of follow-up. The DASH diet appeared to have a positive impact in all studies, with RRs between 0.41 to 0.86 for high adherence versus low adherence. For two trials, the association was statistically significant [93 -95].

**Alkaline diet**

A high concentration of alkaline ions from food that the body receives as a result of metabolic activities define the AD [96,97].According to K/DOQI recommendations, sodium citrate or sodium bicarbonate are administered as an alkali therapy to patients whose serum bicarbonate levels are below 22 mmol/L [98]. Such treatment aims to raise bicarbonate levels and slow the fall of GFR. Unfortunately, there are certain adverse effects to this kind of intervention. In actuality, sodium bicarbonate and sodium citrate both have the potential to boost the stomach absorption of aluminium. Furthermore, substances high in sodium can increase water retention, which can lead to arterial hypertension [99]. Further, the PRAL and potassium content of various foods can be used to build an AD for CKD patients. Several studies have shown that consuming more fruits and vegetables, along with neutralising the dietary acid load with alkali, can slow the course of CKD as measured by kidney damage biomarkers [100,101].It is believed that an increase in acid load causes an increase in endothelin and angitensin II,[102,103]. which accelerates the course of CKD. As a result, the activation of the RAAS speeds up the progression of CKD by producing hyperfiltration and hypertrophy of the residual nephrons as well as damage to the tubule interstices and

glomerulosclerosis. Chronic low-grade inflammation and oxidative stress, both of which are common in CKD patients, further intensify this process [104].Another benefit of AD in CKD patients is its ability to positively modulate gut flora, which results in an increase in the production of short-chain fatty acids (SCFAs) and a decrease in the release of nephrotoxic chemicals like p-cresol as well as indoxyl sulphate [103, 105], Now to sum up in short, the AD is an alternative nutritional therapy for CKD patients.

## CONCLUSIONS

The prevention and treatment of CKD still heavily rely on diet. Despite the fact that there are recognised guidelines for protein intake in CKD patients, new research suggests that the kind of protein consumed may be significant, with a switch from animal to plant sources of protein appearing to be advantageous. Additionally, plant foods include more dietary fibre, and there is mounting proof that increasing dietary fibre and non-digestible carbohydrate intake improves biochemical indicators in CKD patients. Another probable reason by which plant protein appears to be more advantageous when compared to animal sources of protein is the reduced organic phosphate bioavailability found in plant protein meal sources. Restricting sodium and potassium intake is currently advised for CKD patients. The consumption of fruits, vegetables, potassium-rich foods, dietary fibre, and plant proteins has thus generally been limited in CKD patients. There is growing evidence that suggests a diet rich in fruits and vegetables during the early stages of CKD may slow the disease's progression; however, further research is needed before guidelines are changed. Reducing intake may be advantageous for people who consume a lot of sugar-sweetened beverages and have a high dietary fructose intake.To thoroughly establish the impact of these dietary variables in CKD patients, additional high-quality research are necessary. To provide a more comprehensive, evidence-based approach to the dietary management of chronic kidney disease, more research is required.

**Future Scope**

Nutrition diet has a bright scope in development of our body. It helps us to fight against various disorder and maintain our body fit and fine.

## List of abbreviations

| | |
|---|---|
| CKD | Chronic Kidney Disease |
| NICE | National Institute for Health Excellence |
| KDOQI | Kidney Outcomes Quality Initiative |
| GFR | Glomerular Filtration Rate |
| CVD | Cardio Vascular Disorder |
| MDRD | Modification of Diet in Renal Disease |
| REPs | Renal Epo- Producing cells |
| SBP | Spontaneous Bacterial Peritonitis |
| CVE | Cardio Vascular Event |
| UNESCO | United Nations Educational, Scientific and Cultural Organization |
| MUFAs | MonoUnsaturated Fatty Acids |
| DASH | Dietary Approaches to Stop Hypertension |
| RAAS | Renin Angiotensin Aldosterone System |

## DECLARATIONS

**Ethics approval and consent to participate**

No ethical approval or consent to participate was required for this manuscript.

**Consent for publication**
Yes, all the researchers studied have been duly cited and we have all the open access rights to access these studies.

**Availability of data and material**
Web: https://pubmed.ncbi.nlm.nih.gov/

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**Authors' contributions**
MP complied the paper, worked on English, grammar, and collected information. NE was responsible for filtering the useful information and mechanisms enlisted. DM contributed in the basic idea of paper and collected all data . KC was responsible for all high-quality diagrams, data, and information regarding risk factors. All authors have read and approved the manuscript.

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**Table 1 Allopathic drugs used in treatment of chronic kidney failure [21].**

| Class | ***Drugs*** |
|---|---|
| Analgesics | Pethidine |
| Antibiotics | Cefepime |
| Phase- prophylactic psychotropic drugs | Lithium |
| Anti diabetics drugs | Metformin, Glibenclamide, Gimepiride |
| Diuretics | Spironolactone, Eplerenone |
| Immune Suppressants | Methotrexate |
| Radiological contrast media | Gadolinium |
| LMW- Heparin | Enoxaparin |

**Table 2** Nutritional content for patients with CDK [82]

| **Dietary constituent** | **Diet (variables)** | **Baseline eGFR$_{CR}$, mL/min/1.73 m$^2$** | **Findings** |
|---|---|---|---|
| **Protein** | Protein intake (Divided into quintiles) | Normal: > 80 Mild CKD: 55–80 | A high-protein diet was not linked to a decline in eGFRcr in people with healthy kidney function. In mild CKD, it was connected to accelerated |

| | | | |
|---|---|---|---|
| | | | eGFRcr decline. |
| | Protein intake (Divided into quartiles) | Mean 93.9 ± 14.1 | An elevated risk of kidney hyperfiltration and a quick decline in kidney function was associated with a high protein diet. |
| | Low protein diet (0.6 g/kg/day) compared to the typical protein diet (1.3 g/kg/day) | 25–55 (mean 38.6) | At 3 years, the mean eGFRcr decline was the same for both diet groups. |
| | 0.6 g/kg/day of low protein vs. 0.3 g/kg/day of very low protein with ketoacid | 13–24 (mean 18.5) | A very low protein diet with supplements slightly slows the loss of eGFRcr. |
| | 0.6 g/kg/day of low protein vs. 0.3 g/kg/day of very low protein with ketoacid | < 30 (mean 18.0) | The risk of CKD progression was reduced by supplementing with a very low protein diet. |
| **Sodium** | Urinary sodium | Mean 68.4 ± 17.6 | The risk of CKD progression was not increased by urinary sodium excretion. |
| | Urinary sodium | 41.5–48.5 | Increased risk of CKD progression was linked to higher urinary sodium excretion. |
| **Potassium** | Urinary potassium | 41.5–48.5 | Increased risk of CKD progression was linked to higher urinary potassium excretion. |
| | Urinary potassium | 32.6 | Lower risk of death from any cause was associated with higher urine potassium excretion, but not kidney failure. |
| **Phosphorus** | Urinary phosphate | 33 | The risk of KF and mortality is not correlated with higher urinary phosphate excretion. |
| | Prescribed dietary phosphate | Hemodialysis | In patients receiving frequent hemodialysis, dietary phosphate restriction is not linked to a higher survival rate. |
| **Calcium** | Dietary calcium | 54.8 | To avoid calcium deficiency and calcium loading, the daily recommended total intake of elemental calcium should be between 800 and 1,200 mg. |
| **Vitamin D** | Ergocalciferol | Hemodialysis | Ergocalciferol taken orally has the ability to raise 25(OH)D levels without significantly changing blood calcium, phosphate, or parathyroid hormone levels. |
| | Cholecalciferol | 34.6–35.8 | In nondiabetic early CKD, cholecalciferol supplementation to correct vitamin D deficiency has positive effects on vascular function. |

**Table 3 Nutritional Recommendation for Patients [82]**

| Dietary constituent | Nutritional recommendations |
|---|---|
| *Protein* | A protein intake of 0.6-0.8 g/kg/day is advised for patients with CKD G3b, G4, or G5 or patients with proteinuria (urinary protein excretion > 0.3 g/day). Dialysis patients should take between 1.0 and 1.2 g/kg/day. For every 1 g of urinary protein excretion over 5 g per day, patients with nephrotic syndrome should consume 0.8 g/kg/day plus 1 g/day of protein. 0.8-1.0 g/kg/day is advised for patients with nonproteinuric CKD G1, G2, elderly patients with CKD G3b, and those whose CKD is slowly progressing. |

| | |
|---|---|
| *Sodium* | It is advised to consume less than 2 g of sodium (or about 5 g of salt) per day. |
| *Potassium* | It is advised to follow customised regimens to keep serum potassium levels within the normal range. Consider reducing dietary potassium intake to keep serum potassium levels within the normal range in CKD patients who display hyperkalemia. |
| *Phosphorus* | To keep serum phosphate within the normal range in patients with CKD G3-5 and KF on replacement therapy, 0.8-1 g/day or customised regimens are advised. Consider reducing dietary phosphorus intake to keep serum phosphate levels in the normal range in CKD patients who show hyperphosphatemia. Phosphorus intake from vegetables should be increased, and processed food should be avoided at all costs. |
| *Calcium* | To maintain normal calcium levels in CKD G3, G4 patients who are not taking active vitamin D analogues, 800-1,000 mg/day of elemental calcium may be prescribed. Adjust calcium intake (dietary calcium, calcium supplements, or calcium-based binders) in KF patients receiving replacement therapy according to the concurrent use of vitamin D analogues and calci-mimetics to prevent hypercalcemia. |
| *Vitamin D* | Vitamin D supplementation in the form of cholecalciferol or ergocalciferol may be considered in CKD patients who show signs of 25(OH)D deficiency only for deficiency/insufficiency, not for CKD-MBD or other clinically relevant outcomes. Individualized plans for vitamin D supplementation should be made, and serum levels of calcium, phosphorus, and 25(OH)D should be checked periodically, particularly in patients taking calcium-containing phosphate binders and/or active vitamin D analogues. |

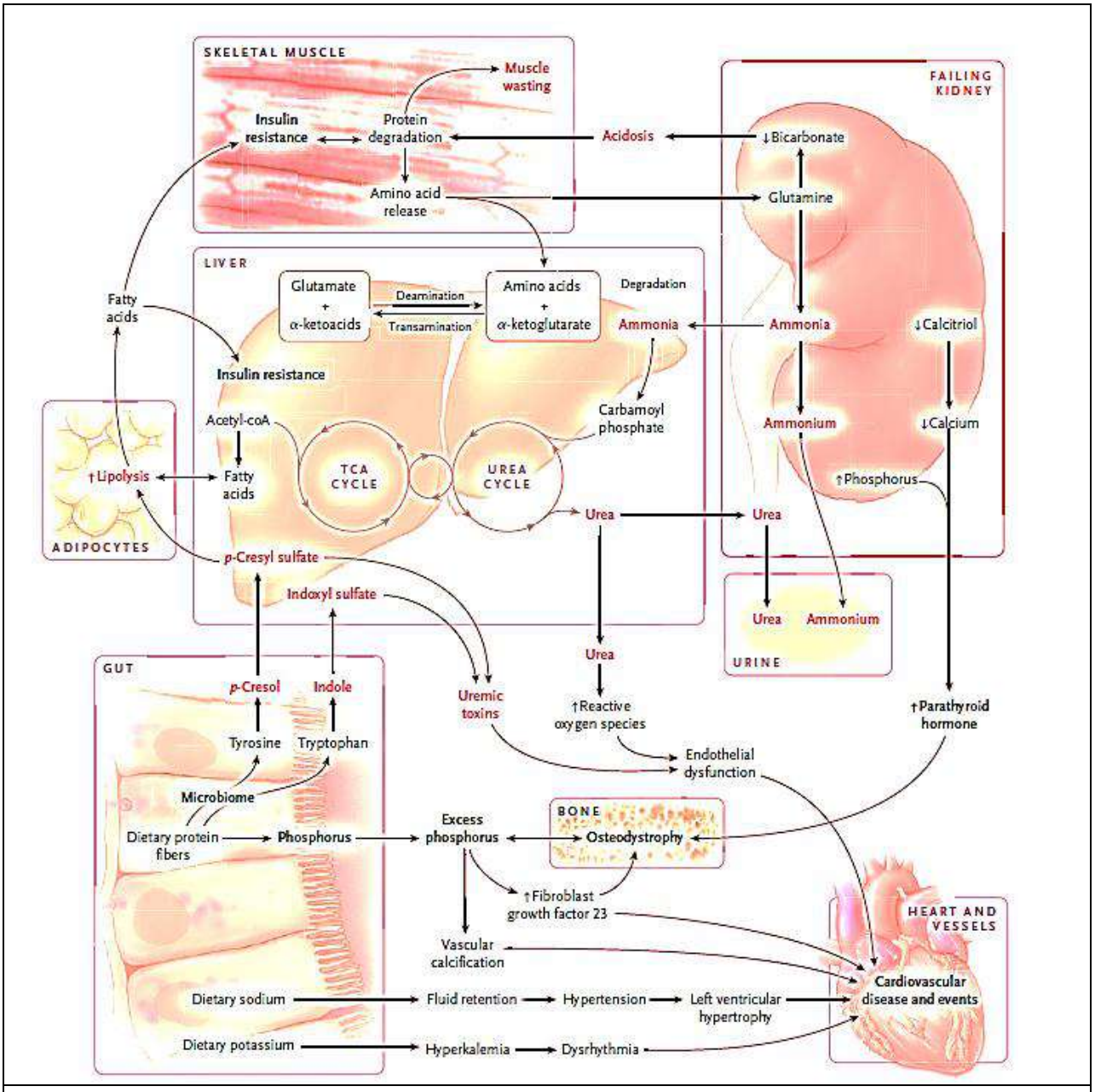


**Fig. 1 Protein and Amino Acid Metabolism Pathway [81]**

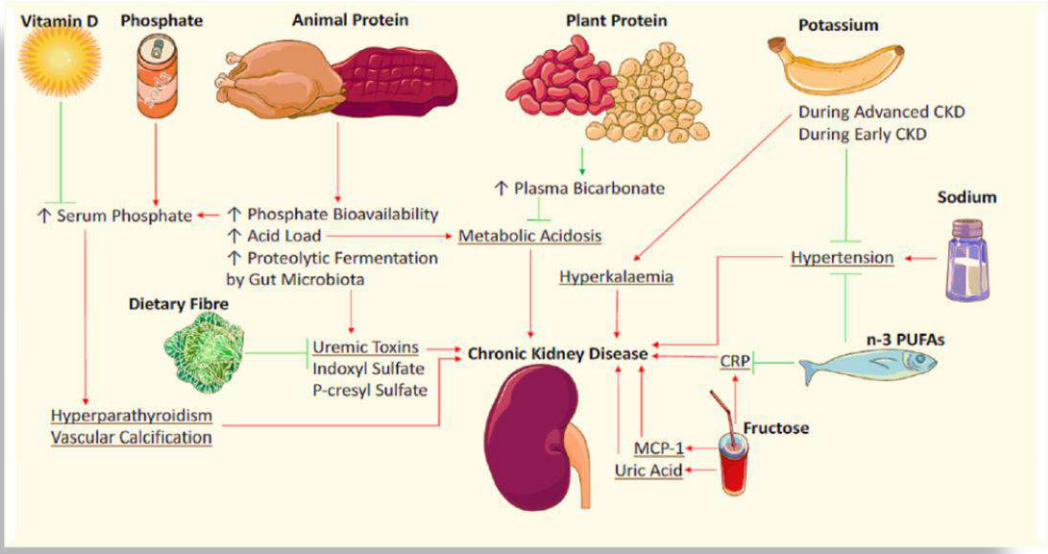


**Fig. 2 . Mechanism of Dietary Factor [83]**

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**RESEARCH ARTICLE**

# The Heavy Metal Pollution is Detected by Fish as a Bioindicator

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## ABSTRACT

As a result of their toxicity, persistence, bioaccumulation, and biomagnification properties, heavy metal pollution poses a significant threat to the environment. The environment may become contaminated with heavy metals from a variety of anthropogenic and natural sources. The primary anthropogenic sources of heavy metals are industrial and agricultural processes, the combustion of fossil fuels and gasoline, waste incinerators, mining, and volcanic eruption and weathering of metal-bearing rocks, among other things. These heavy metals' mobilisation into the aquatic ecosystem changes the physicochemical features of water, endangering aquatic organisms. After a fish ingests diet that has stored metals, the digestive tract, body surface, and gills are the main entry points for heavy metals. The maximum recurrent heavy metal contaminants that produce extreme toxicity in fish include copper, zinc, chromium, nickel, cobalt, iron, aluminium, cadmium, mercury, arsenic, and lead. The key chemical mannerbasic metal toxicity is the increase of oxidative stress. Stress impairs reproductive capacity, damages tissue and organs, down the immune system, and results in growth defects. The necessary trace elements and micronutrients, crucial for growth and metabolism, are particularly copper, zinc, chromium, nickel, cobalt, and iron. The abundance of high-quality protein finds in fish, beside with its high vitamins & omega-3 fatty acid content, boosts societies to eat fish as a highest source of nutrition. As an end result, built-up heavy metals in fish tissues are directly transferred to humans, where they have toxic defects that speed the increase of numerous diseases. Hence, in order to implement the laws and regulations regulating their preservation in the water ecosystem and similarly to preserve human lives, it is vital to explain the foundations of heavy metals and their detrimental influence on fish health.

**Keywords:** Fish; bio – indicator; heavy metals; bioaccumulation; changes; water pollution.

# INTRODUCTION

One of the main issues facing modern human society is environmental degradation [1]. Environmental contamination is on the rise due to the quickly expanding industry, rising energy consumption, and careless depletion of natural resources over the past few decades [2]. Diverse organic and inorganic hazardous substances are continuously introduced into the aquatic environs from together natural and manmade sources. As an end result of both their toxicity and the propensity for bioaccumulation within the food chain, heavy metals play an important role in ecological contamination [3]. The chief sources of heavy metal emissions include coal-burning power plants, untreated sewage sludge, metal pipelines, traffic, and industrial output (foundries, smelt, oil refiner, petrochemical facilities, pesticide manufacture, chemical sector). Metals can be classified into two groups: those that are necessary for life and those that are not. The toxicity of non-essential metals, often known as xenobiotic or foreign elements, such as aluminium (Al), cadmium (Cd), mercury (Hg), tin (Sn), and lead (Pb), increases with concentrations and none of their biological functions have been established [84]. On the other hand, essential metals, such as copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), cobalt (Co), and iron (Fe), are recognised to have a significant biological role [85] and are hazardous when present in excessive concentrations or when there are metabolic shortages.

Fishes have various distinct advantages in defining the natural properties of aquatic systems and in evaluating changes to habitats, which makes them the most significant in biomonitors in aquatic systems for the assessment of metal pollution level [79,80]. The fish at the bottom of the aquatic food chain may also store metals and transmit them to people through their diet, resulting in chronic or acute disorders [82]. Animal health may be harmed and their regular physiological functions may be harmed by the biocontamination of poisonous heavy metals in their various tissues [4]. The capacity of a species to reproduce and its rate of survival are both significantly impacted by heavy metal poisoning. Dependent on the species dosage and exposure period, several of them have been informed to be highly oncogenic, mutagen casing, and teratogenic [5]. The purpose of the current article is to quickly discuss the toxicity, impact, and application of several heavy metals as bioindicators on fish health. In this study, both necessary and non-essential heavy metals are examined.

**TOXICITY**

Toxicology refers to the degradation or loss of cell viability and regenerative capability that may arise from the reversible or irreversible disruption of normal metabolic processes. In severe circumstances, the body may lose all of its organs and perish. Due to things like the availability of certain enzymes, the local blood supply, and the organ's capability for regeneration, localised acute toxicity typically affects organs responsible for absorption and disposal. The skin, stomach, liver, intestines, lungs, and kidneys are a few of these.Names for this type of toxicity include nephrotoxicity for the kidneys and hepatotoxicity for the liver, depending on which organ or tissue is harmed.

**TESTING FOR TOXICITY**

A. A dosage threshold is the dose level below which an effect cannot be seen.
B. The effective dosage, or LD50, is the dose that kills 50% of an animal population. Chemical acute toxicity is frequently quantified using the LD50. The acute toxicity decreases with increasing LD50. A substance is said to be potent if it has a low LD50 and is highly poisonous.
C. A lethal concentration, or LC50, is the amount needed to kill 50% of a population.
D. The pharmacological concentration at which a patient experience a 50% increase in response is known as the EC50.
E. The dose that casing a definitedeffect other than mortality in 50% of the animals is known as the ED50 (effective dose).
F. The inhibitory concentration, or IC50, is the level at which the reaction or binding is abridged by 50%.

**THREATENERS TO TOXICITY:**

1. The harmful agent's chemical makeup
2. Concentration and Dose:
   (a) The exposure method
   (c) The duration of exposure
   (c) Exposure time and
   (d) Exposure route
3. Toxicant metabolism,
4. Gender
5. Age

**NECESSARY HEAVY METALS**

**COPPER (Cu)**

Additional sources of copper toxicity comprise the mining industry, sewage sludge industry, plastics industry, electro-plating manufacturing, metal purifying factories, atmospheric deposition, etc. [28,29]. Copper is a crucial micronutrient and trace element that is necessary for the growing and metabolic activity of living things. Copper is a crucial component of several metabolic enzymes and glycoproteins in fish and supplementary animals. Moreover, it is necessary for the neurological system and the manufacture of haemoglobin [30,31]. Nevertheless, copper has a harmful effect on living things at larger concentrations [32]. Freshwater fish become hazardous to copper at concentrations between 10 and 20 ppb [33]. Freshwater fish that were exposed to copper in the water developed oxidative stress symptoms [34]. Fish with chronic copper toxicity have poor development, shorter life spans, lowered immunological responses, and reproduction issues [35]. Apoptosis was induced by copper poisoning in the gills of teleost fish (*Oreochromis niloticus*) [36]. The liver tissue of *Cyprinus carpio* underwent biochemical and morphological alterations after disclosure to $CuSo_4$ [37]. *Oreochromis mossambicus* that had been exposed to copper manifested a rise in RBC amount, haemoglobin leval, and hematocrit rate [38]. This substance disrupts the operation of olfactory neurons and is neurotoxic to fish [39]. Zebrafish larva exposed to copper developed into more sensitive stages than early develop and mature stages and displayed lateral line malfunction [40]. When exposed to copper, the goldfish larvae *Carassius auratus* exhibited a significant rate of physical malformations and mortality [41]. Fish's gills and body tissue have less copper than their liver, which contains the highest percentage [42]. The oxidative metabolism, fat peroxidation, and protein contented in carp muscle were all affected by the trace element's bioaccumulation [43].

**ZINC (Zn)**

In living organisms, zinc (Zn) is an important trace component and micronutrient that is found in nearly every cell, is involved in the synthesis of nucleic acids, and can be found in many enzymes. It is the second most abundant trace element after iron [84]. Zn also plays a role in more complex processes such as cell signalling, the immune system, and neurotransmission [126,127]. At elevated quantities in the water, zinc wastes can be directly hazardous to fish [128], and either zinc alone or more frequently in combination with copper and other metals can have an impact on fisheries [102, 129].The gills are the primary site of waterborne Zn poisoning [127], where the disruption of $Ca2+$ uptake results in hypocalcemia and final mortality [128]. The other end point of toxicity vary between freshwater and marine fish, with survival, growth, reproduction, and hatching being the most prevalent [127]. A target organ for Zn buildup is also thought to be the kidney of the fish [83].

Some freshwater fishes have considerably more active serum transaminases when zinc is present [130]. Chloride cells being pulled away from the underlying epithelium is the first indication of gill injury. Fish with *Oreochromis niloticus* showed pale and clogged gills when expose to zinc sulphate, according to Abd El-Gawad [131]. Through vacuolated epithelial layaring of the gill rakers, the epithelial layer of the gill filaments was hyperplastic and oedematous. Telangiectasis might be seen in the lamellar blood spaces. According to research [132], exposure to zinc causes degeneration and hyperaemia in the ovarymuscle of *Tilapia nilotica.* Finally, innovative techniques for removing contaminants, containing heavy metals after wastewater, must be used to safeguard fish and aquatic systems from

heavy metal pollution. Activated carbon treatments in the form of powder or granules, ozonation, ultraviolet radiation, and reverse osmosis are a few of these [133].

**CHROMIUM (Cr)**

One of the greatest prevalent trace elements in both saltwater and the crust of the earth is chromium [6]. Several anthropogenic sources, such as leather tanneries, metal dispensation, fuelpurifying, fabric production, alloy production, timberconserving, etc., cause chromium toxicity in aquatic ecosystems [7,8]. Chromium toxicity to aquatic organisms depends on a number of biotic parameters, including age, developmental stage, and species type, as well as abiotic features, including pH, temperature, and water alkalinity. Fish that were first exposed to chromium had a variety of behavioural alterations, including erratic swimming, mucus secretion, analteration in body colour, and lack of appetite, among others [9]. *Cyprinuscarpio* was chronically exposed to chromium at a dose of 2-200 mol/L, and this exposure caused cytotoxicity and a reduction in phagocyte and mitogen-induced lymphocyte activation [10]. When exposed to chromium, *Tilapia sparrmanii* had slower blood clotting times, which led to internal bleeding and are issues in pH [11].

Chromium buildup in *Labeo rohita*, an Indian big carp, reduces the amount of total protein and lipids in the tissue, liver, and gills [12]. *Colisa fasciatus*, a freshwater teleost, showed decreased hepatic glycogen upon exposure to chromium [13]. Cr6+ poisoning in rainbow trout, *Salmo gairdneri*, demonstrated respiratory and osmoregulatory failure at pH 7.8 and 6.5 [14]. When Chinook salmon were showing to chromium over an extended period of time, it resulted in physiological anomalies, DNA impairment, microscopic lesions, and decreased growth and endurance rates [15]. Chromium disclosure at a dosage of 2 mg/L in rainbow trout *Salmo gairdneri* impaired egg hatching and fish growth [16]. Different fish tissues exhibit variable chromium bioaccumulation (Table 1). Chromium is accumulated most heavily in the gills, liver &kidney and at extremely low levels in muscle tissue [17].

**NICKEL(Ni)**

Nickel is a fairly common trace element that is found in mishmash with $O_2$ or sulphur in the environment. The environment is exposed to nickel from together natural and human-made sources. When new nickel is mined and converted into alloys or nickel mixtures, the element is released into the environment. Furthermore, nickel is emitted through waste incinerators, oil- and coal-burning power system, and coal-fired power plants [56]. At low concentrations, nickel is a necessary element for many species, but at excessive concentrations, it is hazardous [57]. The physiochemical characteristics of water, such as pH, ionic power, high temperature, hardness, and solidified organic carbon (DOC), among others, affect the poisonousness of nickel to fish [58]. When Nile tilapia were exposed to nickel chloride, they exhibited irregular swimming behaviour, fast opercular movement, respiratory disorders, and skin lesions. The blood parameters of Nile tilapia treated to nickel also changed, with a rise in RBC amount and a decrease in haemoglobin and WBC amounts [59].

Freshwater fish treated to nickel exhibited histopathological variations in various tissues, including the gills, kidney, liver, and intestine. *molitrix hypophthalmichthys*. The liver tissue showed fusion of the gill lamellae, necrosis of the hepatocytes, blood vessel degeneration, enlargement, vacuolation, pyknotic nuclei, and wound. With exposure to nickel, tubular cells in the kidney tissue also showed signs of hyperplasia and degeneration [60].

Nickel exposure in both chronic and acute forms in freshwater fish decrease ATPase action in the brain in *oreochromic niloticus* [61]. exposure to nickel in freshwater fish *Prochilodus lineatus* caused DNA damage to equally blood cells and gills while affecting the liver's antioxidant defence system [62]. The common carp, *Cyprinus carpio,* experienced stress after being exposed to An extraordinary dose of nickel for a brief period of time. In *Cyprinus carpio,* exposure to sublethal nickel concentrations was also reported to alter haematological parameters and cause behavioural abnormalities [63]. *Cyprinus carpio*, a freshwater fish, revealed some negative effects of nickel toxicity on its protein metabolism. Later exposure to a deadly dose of nickel, the detected modifications were a decrease in structural, solvable, and entire proteins, an increase in unrestricted amino acids and protease action, and an increase in ammonia in the gill & kidney [64]. Before dying, fish with nickel poisoning displayed behavioural abnormalities such

surfacing and fast mouth and operculum movement. Nickel builds up in fish blood, kidney, tissue, and liver, with kidney showing the largest accumulation [66]. *Tilapia nilotica's* liver and muscle showed a general decline in glycogen levels as a end result of bioaccumulation. Elevated levels of nickel bioaccumulation lead to lymphopenia and leukopenia in *Tilapia nilotica* by increasing haemoglobin content, packed cell volume, and blood cell count.

**COBALT(Co)**

Cobalt is a crucial ingredient for male health and is a module of vitamin B12; nevertheless, because it interferes with the enzyme system, it also makes fish harmful in vital aquatic ecosystems [134]. It has recently been included to group 2A carcinogens due to reports that it may be a potentially cancer-causing substance (i. e., probably carcinogenic to humans). The amount of cobalt that is permitted to be present at any given time in the air, water, and earth is limited to 1-5 mg/m3, 1 mg/l, and 1 mg/m2, respectively. Cobalt content in rivers is minimal, at just approximately 0.,2 mg/l[135]. The range of the daily average intake of cobalt in humans, in all forms, is 0.30 to 1.77 mg/day [136]. Moreover, it has been connected to controlling blood pressure [137]. and was discovered to be essential for healthy thyroid function [138]. Anemia, polycythemia, and congestive heart failure have all been linked to excessive cobalt use [139].

**IORN (Fe)**

Iron is a common component of mining and industrial effluents, which are frequently released into aquatic habitats. Fish are thought to be more poisonous to ferrous iron ($Fe^{2+}$) than to ferric iron ($Fe^{3+}$) [103]. The liver and gonads of fish had the maximumbio concentration of iron, while the brain, tissue, and heart had the lowest concentration [104,105]. In their work, Omar et al. [83] recently demonstrated that the fish liver is the organ that iron is most likely to target. As a potential mechanism for iron poisoning, physical gill blockage has been suggested as a cause of respiratory disturbance [106]. Solvable ferrous Hg can be converted into unsolvable ferric complexes, which can subsequently coat the gill lamellae and prevent respiration, because the surface of a fish's gills tends to be high pH [107].

Precipitated iron compounds have catastrophic effects on fish, including a reduction in the amount of gill space available for breathing, damage to the breathing epithelium, and eventual suffocation and death. Iron led to secondary lamellae hyperplasia and necrosis in banded tilapia (*Tilapia sparrmanii*) [108]. When exposed to iron, brook charr Salvelinus *fontinalis (Mitchill*) experienced respiratory distress, according to Gonzalez et al. [109], and *Tilapia sparrmanii*, according to Grobler et al. [105], experienced a lessening in action, coughing, yawning, spasmodic actions, and an upsurge in opercular actions (Smith). In agreement with past studies, Peuranen et al. [185] noted iron accumulation on the external of the gill epithelia of brown trout *Salmo trutta L*. that had been exposed to iron. They noted gill injury when exposed to iron concentrations of 0.8–1.7 mg/l at pH levels 5 and 6.

They hypothesised that because iron had only been found on the face and not inside the gill epithelia, it had to operate on the gill face to exhibit its deadly effects. The gills of *T. sparrmanii* collapsed and produced more mucus cells after being exposed to sublethal iron concentration for 72 hours in a continuous flow system, according to a scanning electron micrograph research [104]. Gill collapse lowers the distance at which water and blood diffuse, which helps fish consume less oxygen. Furthermore, iron complexes can impulsive on the surface of fish eggs, leading to oxygen deprivation and eventual death [90]. When given a diet high in Fe, *Clariasgariepinus* displayed constrained growth [111].

**UNNECESSARY HEAVY METALS**

**ALUMINUM (Al)**

After silicon and oxygen, aluminium (Al) is the third greatest prevalent and plentiful metal on earth [78]. It is comparable to numerous other metals in that its soluble ionic form is typically thought to be the most hazardous [87]. It is utilised as a flocculation agent in water management and can be detected in the air of large cities and industrialised areas [88,89]. Aluminum's toxicity to fish is significantly influenced by the physicochemical character of the water, particularly by its pH. At pH levels lower than 6.0, aluminium is soluble [90]. The reason of fish

poisoning has been discovered to be significant union of lamellae and filaments in the gills, intrusion with ionic and osmotic equilibrium, and respiratory issues as a result of mucous coagulation on the fish's gills [91]. In females of the *Oreochromisniloticus* species that are mature, al is regarded as an endocrine disruptive substance [92]. Fish expose to Al had significantly smaller indicate corpuscular volume (MCV) and significantly greater mean corpuscular haemoglobin concentration (MCHC), hematocrit (Hct), and indicate corpuscular haemoglobin (MCH) [93]. Fish development was significantly reduced when aluminium concentration as low as 0.52 mg/l were used [94]. In addition to structural gill damage [101], common physiological change in fish species exposed to Al include abnormalities in the cardiovascular [95], hematologic [96], respiratory, ion regulatory [97], reproductive [98], metabolic [99], and endocrine [100] systems.

**CADMIUM(Cd)**

The average quantity of cadmium in the earth's scab is amongst 0.1 and 0.5 ppm, and it is frequently associated with the ores of zinc, copper, & lead. The concentration is typically between 1 and 5 mg/L in surface and ground water, and between 5 and 110 mg/L in ocean water. Cadmium does not exist in element form in nature. Instead, chemical forms of cadmium, such as cadmium oxide, cadmium sulphate, and cadmium chloride, are used. Cadmium cyanide, nitrate, and carbonate are frequently observed. Many anthropogenic and normal sources transfer cadmium into the aquatic ecosystem. Cadmium is naturally obtained from the earth's scab and covering through volcanic eruptions and rock weathering. Because it is a non-essential metal, cadmium is extremely hazardous to fish. *Cyprinuscarpio* experienced DNA damage as a effect of low-level cadmium exposure [18]. Cd+2 was discovered to limit trans-epithelial calcium influx in the rainbow trout gills [19].

Fish exposed to subchronic levels of cadmium chloride developed micro- and bi-nucleated cells in their blood, gills, and liver [20,21]. Hepatocyte necrosis, congestion of submucosal blood vessel in the gut, and glomerular reduction and necrosis in the kidney tissue of Tilapia are just a few of the reported histological changes (*Oreochromisniloticus*). Fish that had been exposed to cadmgg1ium shown a varied haematological reaction. American eel fish ( *Anguilla rostrata* ) exposed for 8 weeks to 150 g/L of cadmium develop anaemia as a effect of decreased heamoglobin and erythrocyte count. After cadmium exposure, a significant rise in leukocyte and big lymphocyte counts was also seen [22]. When *Cyprinuscarpio* was subjected to sublethal concentration of cadmium, the stage of glycogen distance in the muscle and liver was dramatically reduced, and the blood glucose level rose [23]. Inhibiting vitellogenesis and an endocrine disruptor, cadmium has been initiate in rainbow trout Oncorhynchus mykiss [24]. Common carp *Cyprinuscarpio's* gonad purpose and sexual development were both impacted by cadmium chloride exposure [25]. *Leuciscusidus* larvae exposed to cadmium exhibited body abnormalities and a lower incidence of embryonic survival attributable to mortality in newly emerged larvae [26]. As a result of its slow rate of emission, cadmium buildup poses a major threat to the environment. The epidermis has the lowest range of cadmium bioaccumulation, whereas the liver, kidney, & gills have the highest levels. The gill is the organ that detoxifies cadmium the fastest [27]. Due to its rapid rate of bioaccumulation, cadmium is one of the greatest dangerous heavy metals for water creatures .

**Mercury (Hg)**

Fungicides, particularly organic fungicides that contain mercurial components, which are organic mercury compounds, are the primary source of mercury in the ecosystem. The chronic data on mercury toxicity showed that methylmercury (MeHg+), an organic form of mercury, is the mercury component that is most dangerous over the long term [112, 113]. It is also believed that 70–100% of the mercury in fish is contained as MeHg+ [114, 115]. Fish tissues have a high ability for both organic and inorganic forms of mercury to accumulate, making them perceptive indicators of water contamination [117].

Although injuries to the gill arches, liver, kidney, blood parameter, olfactory epithelium , and nervous system have been noted [118,120], some studies have exposed that mercury compounds can remaining the tissues of animals for extended periods of time, causing irreversible injuries like neurological destruction and lesion, behavioural and cognitive change, ataxia, as well as convulsion, in addition to its detrimental effect on reproduction [121,122]. There have been previous reports of renal tubular lumen necrosis and fibrosis in *Clariasbatrachus* kidney subjected to

chronic mercury [123]. Mercury affects the viability of spermatozoa, egg formation, and the endurance rate of developing eggs and fry at very low concentration [124]. Zaki et al. [125] found that fish fed with 15 mg/kg diet mercuric oxide for 4 weeks had significantly higher levels of cholesterol, alkaline phosphatise, alanine amino transferase (ALT), aspartate amino transferase (AST), and cortisol, and significantly lower levels of haemoglobin (Hb), hematocrit (Ht), indicate total protein value in serum, and body mass.

**ARSENIC (As)**

Arsenic is a common element that is released into the aquatic environment via a variety of human-made sources, such as manufacturing firms, smelting activities, power plants, etc. The apply of arsenic insecticides, herbicides, and fungicides in agricultural fields is a significant additional source of arsenic [67]. Fish ingest arsenic-contaminated food, which also exposes them to arsenic-contaminated water throughout their gills and skin. There are several different forms of arsenic, including the elemental, trivalent, & pentavalent oxidative forms. The trivalent oxidation state of inorganic arsenic (arsenites) is more poisonous than the pentavalent status and is absorbed into fish tissue very quickly (arsenates). Arsenic's toxicity is influenced by a number of abiotic parameters in water bodies, including pH, temperature, salinity, and organic matter, phosphate concentration, suspended particles, and other toxins [68]. Freshwater fish that are constantly exposed to low levels of arsenic develop bioaccumulation, primarily in the liver and kidney tissue [69]. Freshwater fish, including tilapia, revealed histological changes after exposure to arsenic (*Oreochromis mossambicus*). Epithelial hyperplasis, lamellar mixture, epithelial lifting and oedema, desquamation, and necrosis were the modifications in the gills. The histology of the liver revealed macrophage infiltration, vascularization, shrinkage of the hepatocytes, enlargement of the sinusoids, vascular disintegration, nuclear hypertrophy, and localised necrosis [70]. Freshwater teleost *Channapunctata'*sheart was found to have a variety of histological changes, include necrosis in the heart tissue [71].

The erythrocyte membrane was ruptured, hemolysis, poor iron uptake by erythrocytes, and disrupted haemopoiesis in common Indian catfish *Clarias batrachus* after acute experience to sodium arsenite [72]. The total leucocyte count changed over time in the catfish *Clarias batrachus* expose to arsenic, and the oregano-somatic indicators in the kidney and spleen decreased. Moreover, arsenic altered T- and B-cell function and interfered with catfish's ability to phagocytose microorganisms [73]. The Japanese medaka (*Oryzias latipes*) rudiment experienced developmental arrest at sublethal levels of arsenic poisoning [74]. When exposed to arsenic, rainbow trout *Salmo gairdnerii* developed stress response proteins [75]. In zebrafish embryos, arsenic poisoning greatly reduces the expression of genes implicated in natural immune response, which protect against bacterial and viral infection [76]. Arsenic oxide exposure for a long period of time resulted in decreased ovarian function and a decline in the improvement of 2nd and 3rd stage oocytes in fresh water fish *Colisa fasciatus* [77]. Fish physiological systems such as development, reproduction, gene appearance, ion control, resistant system, and histopathology are all impacted by arsenic bioaccumulation.

**LEAD(Pb)**

For the most part dangerous heavy metals is lead, which is found in nature in arrangement with other rudiments such as PbS, PbSO4, and PbCO3. Several anthropogenic source, such as lead-arsenate insecticides, lead-based paint, pigment, food can, and the incineration of coal, oil, and gasoline, among others, significantly increase the absorption of lead in the atmosphere [44]. The aquatic environment is immediately impacted by lead discharge from a variety of industrys, farming fields, street excess, lead dust, and municipal waste water, which toxicity for aquatic life [45]. pH, salinity, hardness, and other factors all affect how soluble lead is in water. In flexible and acidic water, lead dissolves most readily. Lead poisoning in fish can occur at concentrations of 10-100 mg/L [46]. Fish exhibit altered behaviour, impotence, and slowed growth when expose to sublethal levels of lead [47]. After extended experience to a low absorption of lead nitrate, Katti found that the liver, brain, & gonad of *Clarias batrachus* had changed in lipid and cholesterol content [48]. African catfish *Clarias gariepinus* covered to lead showed histological deformation of the gill and liver tissue. Fresh water teleost (*Mastacembelus pancalus*) exposed to lead displayed histological changes in the ovarian tissue [49].

Fish exposed to lead also showed signs of parenchyma cell necrosis, hepatic cord and connective tissue fibrosis, decreased growth and body weight, and blood vessel collapse [50]. Reduced haemoglobin pleased, red blood cell count, and hematocrit values were seen in Nile tilapia (*Oreochromis niloticus*) after lead exposure [51]. Lead toxicity causes synaptic damage and neurotransmitter dysfunction in fish, which leads to oxidative stress [52]. Lethal and sublethal disclosure to direct in Tench (*Tincatinca*) led to changes in immunological markers [53]. The liver, spleen, kidney, & gills they all are the primary sites of direct bioaccumulation in fish [54]. *Acipensersinensis*, a species of Chinese sturgeon, experienced morphological changes and decreased free movement as a effect of lead bioaccumulation [55].

## CONCLUSION

In conclusion, this study has shown that heavy metals have a detrimental effect on fish. It is extensively clear that metals cause an untimely response in fish, as evidence by changes in the structural and functional characteristics of various organs, together with enzymatic and genetic defects. This affects the fish's innate immune system and makes them more vulnerable to a variety of diseases. Biomarkers are a useful tool for developing policies for efficient environmental management since they can provide additional physiologically and ecologically pertinent information. Fish biomarkers are therefore essential for tracking environmental changes and determining how xenobiotic substances (such as heavy metals) affect fish. It is also advised that all waste water, sewage, and agriculture wastes be treated earlier being released into the aquatic systems. The implementation of all legal provisions and legislation pertaining to the preservation of aquatic habitats must also be taken into account.

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**TABLE: 1**

| S.NO. | HEAVY METALS | FISH SPECIES | BIOACCUMULATION INORGAN OR TISSUE | REFERENCE |
|---|---|---|---|---|
| 1 | Copper(Cu) | *Oreochromis niloticus*<br>*Cypronus carpio*<br>Zebrafish larva | Apoptosis<br>Biochemical and morphological changes<br>Latreal line dysfuntion | Varanka Z, 2001<br>Varanka Z 2001<br>Johnson A, 2008 |
| 2 | Zinc(Zn) | *Oreochromis niloticus*<br>*Tilapia nilotica* | Histological alteration<br>Affect ovarion growth | Abd El-Gawad AM,1999<br>Carino VS, 1990 |
| 3 | Chromium(Cr) | *Tilapia sparrmanii*<br>*Labeo rohita*<br>*Cyprinus carpio* | Hematological alteration<br>Biochemical changes in tissue<br>Histological alteration | Van pittius MG, 1992<br>Vutukuru SS, 2003<br>Yeilbudak B, 2014 |
| 4 | Nickel(Ni) | *Hypophthalmichthys molitrix*<br>*Cyprinus carpio* | Histological changes in defferent tissue<br>Hematological alteration | Athikesavan S, 2006<br>AL-Ghaim KA, 2011 |
| 5 | Cobalt(Co) | *Danio rerio* | Behavioral changes | Chandrabhushan singh, 2017 |
| 6 | Iron(Fe) | *Tilopia sparrmanii*<br>*Tilopia sparrmanii*<br>*Salmo trutta* | Histological alteration<br>Hematological alteration<br>Behavioral changes | Van Rensburg EL, 1989<br>Grobler VHE, 1991<br>Peuranen S, 1994 |

| | | | | |
|---|---|---|---|---|
| 7 | Aluminum(Al) | *Oreochromis niloticus*<br>*Tilapia zillii*<br>*Leporinus macrocephalus* | Endocrine disorder<br>Heamological alteration<br>Heamotological alteration | Correia TG, 2010<br>Alwan SF, 2009<br>Barcarolli IF, 2004 |
| 8 | Cadmium(Cd) | *Anguilla rostrate*<br>*Cyprinus carpio*<br>*Cyprinus carpio* | Heamotological alteration<br>Biochemical alteration<br>Affect gonad function | Gilkl TS,1993<br>Cicik B, 2005<br>Das S, 2013 |
| 9 | Mercury(Hg) | *Clarias batrachus*<br>*Clarias gariepinus* | Hematological alteration<br>Biochemical alteration | Kirubagaran R, 1988<br>Zaki MS,2011 |
| 10 | Arsenic(As) | *Oreochromis niloticus*<br>*Oreochromis mossambicus*<br>*Clarias batrachus* | antioxidant enzyme alteration<br>histological alteration<br>heamatological alteration | Min E, 2014<br>Ahmed MK, 2013<br>Tripathi S, 2003 |
| 11 | Lead(Pb) | *Tinca tinca*<br>*Clarias gariepinus* | Affect immunological parameters<br>Histological alteration | Shah SL, 2005<br>Olojo EAA, 2005 |

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**RESEARCH ARTICLE**

# Deciphering Molecular Docking Studies of Phytoconstituents Identified in Polyherbal Siddha Formulation *Sathakuppai Kudineer* on HMG coA Reductase – An Enzyme Target for Anti-Hyperlipidemic Activity

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## ABSTRACT

Hyperlipidemia, in particular elevated LDL (hypercholesterolemia), is one of the most prevalent risk factors contributing to the evolution of atherosclerosis & consequent vascular disease. This condition is treated with lipid-lowering medications & diets, as well as exercise & lifestyle modifications. Statins, also known as HMG-CoA reductase inhibitors, are the medications most frequently used to treat hyperlipidemia. Statins are generally regarded as safe & tolerable medications, but certain disagreements persist. Molecular docking study holds great potential in Siddha medicine, particularly in the context of herbal formulations. The phytoconstituents of the medicinal plants were retrieved from the PubChem chemical database. The target for the docking study was selected as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA reductase). *In-silico* docking analysis was performed by using Auto Dock Vina. The Auto dock program was used to evaluate the different orientations of the lead molecules with the target protein, & based on the interaction analysis, the best dock pose was chosen. According to the results of Computational analysis, the plants used in the Siddha formulation *Sathakuppai kudineer* yielded a total of 11 bioactive lead components. The phytochemicals such as Chlorogenic acid, Friedelin, Betulinic

acid, Daucosterol, Gingerdione, &Piperlongumininepresent in the herbal ingredients reveal maximum interactions with HMG CoA Reductase when compared to that of Atorvastatin. Therefore,it was concluded that these bio compounds may exert good anti-hyperlipidaemic activity & thereby considered a drug of choice for the clinical management of hyperlipidemia. Further preclinical studies & clinical trials must be carried out before the clinical recommendation of the *Sathakuppai kudineer* to the above-mentioned indication.

**Keywords:** hyperlipidemia, *Sathakuppai kudineer* , Molecular docking, HMG CoA reductase

## INTRODUCTION

Hyperlipidaemia is a very common disorder that is used to describe various inherited & acquired disorders, characterized by high lipid levels in the body. Hyperlipidaemia is classified into two broad classifications: primary (inherited) & secondary (acquired) hyperlipidemia. Primary hyperlipidemia is caused by an array of genetic disorders that a patient may inherit at birth, whereas secondary hyperlipidemia derives from an underlying etiology, such as central obesity, hypothyroidism, uncontrolled diabetes, unhealthy diet, medications (amiodarone, glucocorticoids) & poor lifestyle[1-2].According to estimates from the World Health Organisation (WHO), high blood cholesterol results in over 4.4 million deaths annually & accounts for approximately 56 percent of cases of cardiovascular illnesses globally[3].Complications of untreated hyperlipidemia cause complications like coronary artery disease (CAD), cerebrovascular accidents (CVD), strokes, peripheral artery disease ( PAD), & even death. Patients with hyperlipidemia are more prone to develop cardiovascular disease (CVD).Numerous preclinical & clinical studies have proven that inhibiting the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), a crucial enzyme in the synthesis of cholesterol, can reduce the risk of coronary problems [4].Statins & other well-known drugs (niacin, fibrates, etc.) work by suppressing HMGCR activity,either by inhibiting endogenous synthesis or by lowering cholesterol absorption from the intestine to treat hyperlipidemia[5]. Currently, available hyperlipidaemic drugs have adverse effects like nausea, diarrhea, gastric irritation, hyperuricemia, myositis, & abnormal liver function[6]. In recent years, researchers have turned their attention to medicinal plants to investigate phytochemicals with HMGCR inhibitory activities that could be used to develop a new hypolipidemic drug with little or no side effects.

Computational methods have been widely used in evaluating the therapeutic potential of medicinal plants by determining their activity against the target protein as well as their possible mechanism of action as technology has advanced. Molecular docking techniques predict interactions of protein & ligand with their binding affinities & the binding orientation of protein-ligand complexes. A ligand's molecular structure, orientation, & conformation are related to its ability to bind to a specific protein. Energy-based scoring & geometric sampling are the two steps involved in docking methods that determine the ligand/protein binding affinity. Based on the scoring function, the best complementary protein-ligand binding is found depending on their predicted binding affinities. High energy scores are directly related to protein-ligand binding affinity [7-12]. India is renowned for its extensive collection of living ethnomedical traditions as well as its rich traditional medical systems, including Ayurveda, Siddha, & Unani[13]. In traditional Siddha medicine(TSM),numerous herbal formulations are useful in treating hyperlipidemia.*Sathakuppai kudineer* (SK)is a classic Siddha Polyherbal formulation that is mentioned in the Siddha literature Siddha formulary of India.This poly herbal drug contains nine ingredients like *Omam, sathakuppai, moongil, mavilingam, parangipattai, chukku, thippili, koduveli, &karunjeerakam*[14].The lead molecules were analyzed for their ability to block the HMG CoA reductase from a broader scientific perspective. This may help in the acceptability of SK as an Anti-hyperlipidaemic agent.The study aims to evaluate an in-silico computational analysis of the phytochemicals found in *Sathakuppai kudineer* on HMCoA reductase- an enzyme target for antihyperlipidemic activity.

## MATERIALS AND METHODS

Docking calculations were done for the compounds retrieved from herbal drugs such as d-carvone,Thymoquinone, p-cymene, Chlorogenic acid, Friedelin, Betulinic acid, Daucosterol, Plumbagin, Gingerdione, Piperlonguminine, (-)-beta-Bourbonene, & standard Atorvastatin against target protein (Table.1 &FigA). The ligand molecular properties are depicted in Table. 2.

**Target Protein Retrieval**

The binding of phytocomponents with the core amino acids (Ser684, Asp690, Lys691, Lys692) of the target by forming a hydrogen bond will hinder the function of the enzyme HMG CoA reductase with PDB – 1HWK. These amino acid residues are functionally responsible for HMG CoA reductase enzyme activity in cholesterol biosynthesis. Thereby phytocomponents that inhibit the target enzyme HMG CoA reductase may occupy these active amino acids&could be able to block the HMG coA enzymatic activity thereby limiting the cholesterol synthesis & subsequently helping in control the hyperlipidemia. Compounds that selectively hinder the action of this enzyme may act as a potential therapeutic agent for the management of hyperlipidemia.The crystalline structure of the target protein HMG coA reductase(1HWK) was retrieved from the protein data bank (RSCB) & the essential missing hydrogen atoms were added after the protein cleanup process. The Auto dock program was used to evaluate the different orientations of the lead molecules in relation to the target protein, & based on the interaction analysis, the best dock pose was chosen.

**Molecular Docking Methodology**

Docking calculations were carried out for retrieved phytocomponents against target enzyme Phospholipases A2. Essential hydrogen atoms, Kollman united atom type charges, & solvation parameters were added with the aid of Auto Dock tools[26-27]. Affinity (grid) maps of ×× Å grid points & 0.375 Å spacing were generated using the Auto grid program. Auto Dock parameter set- & distance-dependent dielectric functions were used in the calculation of the van der Waals & the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) & the Solis & Wets local search method [28]. The initial position, orientation, & torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, & quaternion & torsion steps of 5 were applied.

## RESULTS AND DISCUSSION

The Summary of the molecular docking studies of the lead compounds & Standard Atorvastatin against HMG CoA reductase& its amino acid binding interactions have been tabulated in Table No. 3, 4, & Fig C.Hyperlipidemia is a major risk factor for the development & progression of atherosclerosis & coronary heart disease. It is related to risk factors like arteriosclerosis, hypertension, type-II DM, obesity, MI, congestive cardiac failure, angina pectoris, gall bladder diseases, degenerative joint diseases, apnoea, & sterility[29].Complications from undertreated or untreated hyperlipidemia include all types of vascular disease, like coronary artery disease, peripheral artery disease, cerebrovascular accidents, aneurysms, type II diabetes, high blood pressure, & even death[30].Currently, the most widely recommended lipid-lowering medications to lower plasma lipids are statins, including atorvastatin, lovastatin, & simvastatin[31].Hypolipidemic drugs (statins) are structural analogues of HMG CoA, but inhibitors of HMGCR (HMG-CoA reductase).Target protein HMG-CoA reductase catalyses the reductive conversion of HMG-CoA into mevalonate, a rate-regulatory step that regulates endogenous cholesterol biosynthesis[32].This results in a compensatory increase in LDL receptor-mediated uptake & catabolism of IDL(intermediate-density lipoprotein)& LDL (Low-density lipoprotein). Over the long term, feedback induction of HMG-CoA reductase tends to increase cholesterol synthesis, but a steady state is attained with a dose-dependent lowering of LDL-C levels. The drug statins decrease the LDL-Cholesterol by 20-55%, increase the HDL-C levels by 5-15%, & decrease the Triglycerides by 10-

35%[33].The present treatment for hyperlipidemia is Cholesterol-lowering medications may cause life-threatening or serious unwanted effects, such as muscle pain, exaggerated glucose levels, constipation, nausea, diarrhoea, abdomen pain, cramps, & the elevation of liver enzymes[34].People are searching for safer alternatives to these pharmaceuticals because of their adverse effects, & there has been increased interest in finding novel drugs that can lower & regulate blood cholesterol levels resulting in numerous reports on significant activities of natural agents[35]. *Sathakuppai kudineer* (SK), A traditional Siddha polyherbal formulationis used in Clinical Practice by Siddha practitioners to treat irregular menstruation, amenorrhoea, & hormonal imbalances. Nevertheless, the phytoconstituents present in this formulation have proven the Anti-hyper lipidemic, Anti-oxidant, cardioprotective, &Anti-atherogenic activities in various scientific studies. Therefore, the present investigation was designed to investigate the Insilco Molecular docking studies of polyherbal formulation *Sathakuppai kudineer* on HMG Co-A reductase (PBD:1hwk).Based on the molecular docking studies using AutoDock tools, the inhibitory possibility of phytocomponents from *Sathakuppai kudineer* towards HMG CoA reductase (PDB) - 1HWK was investigated with standard Atorvastatin.

Molecular docking is commonly employed as a preliminary step in the development of bioactive compounds that can be used as drug candidates [36]. because it can offer molecular modeling of the interactions between ligands & receptor proteins [37-38]. It is one of the most widely used methods in structure-based drug design (SBDD) due to its ability to predict the conformation of small-molecule ligands within the appropriate target binding site with high accuracy [39]. The In silico docking process is used to select the target protein & reveals the potential role of phytochemicals present in the formulations.Molecular Docking Study is significantly important to find the compounds present in the medicines & their effects on the clinical management of Hyperlipidemia. Furthermore, this approach is significantly less expensive than the traditional method. Prediction in silico is crucial for improving research in pharmacokinetics.

A total of 11 bioactive lead compounds were retrieved from the herbal & other ingredients present in the Siddha formulation. From the reported data of the bioactive compounds, the compounds such as Chlorogenic acid, Friedelin, Betulinic acid, Daucosterol, Gingerdione, &Piperlongumininepresent in the herbal ingredients reveal a maximum of 3-4 interactions with the core active amino acid residues present on the target HMG CoA reductase. Similarly, Compounds like Thymoquinone & plumbagin showed 2 interactions with the active position of the objective enzyme HMG CoA reductase.The results of the binding affinity of these compounds with their respective targets are given in Table No. 3.The Phyto Compounds Beta- Bourbonene&Chlorogenic acid show high affinity such as-6.03 kcal/mol & -5.95 kcal/mol respectively. The Phytocompounds such as Daucosterol, Friedelin, Plumbagin, Betulinic acid, &Thymoquinoneshow more binding potential against the target protein molecules which are –5.88 Kcal/mol, -5.60 Kcal/mol, -5.04 Kcal/mol, -4.92 Kcal/mol, -3.98 Kcal/mol, respectively. The Molecular Docking studies of these Phyto compounds conclude that the drug *Sathakuppai kudineer* possesses promising HMG CoA reductase inhibitory activity & functions as a potential therapy agent for the management of Hyperlipidemia.

## CONCLUSION

Based on the results of the computational analysis it was concluded that the bio-active compounds like Chlorogenic acid, Friedelin, Betulinic acid, Daucosterol, Gingerdione,&Piperlongumininepresent in the ingredients possess significant binding against the target enzyme HMG CoA reductase. Thereby phytocomponents that inhibit the target enzyme HMG CoA reductase by occupying these active amino acids could be able to hinder the function of the limiting enzyme involved in cholesterol biosynthesis.

**Ethical Approval :**
Not Applicable

**Authors Contributions**
Conceptualization, supervision, Methodology, Data interpretation, Writing, & editing done.
**conflict of interest:**
Nil
**source of funding:**
Self

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**Table 1: Ingredients of *Sathakuppai kudineer* with its botanical name & phytocompounds selected for docking**

| S.NO | VERNACULAR NAME | BOTANICAL NAME | PARTS USED | PHYTOCOMPOUNDS |
|---|---|---|---|---|
| 1 | *Sathakuppai* | *Anethum graveolens L.* | Fruit | d-carvone [15] |
| 2 | *Omam* | *Trachyspermumammi L.* | Fruit | p-cymene [16] |
| 3 | *Moongil* | *Bambusa vulgaris Schrad. Ex J.C.Wendl.* | Leaf | Chlorogenic acid [17] |
| 4 | *Mavilingam* | *Crateva religiosa G.Forst.* | bark | Friedelin, Betulinic acid[18-19] |
| 5 | *Parangipattai* | *Smilax china L.* | Rhizome | Daucosterol[20] |
| 6 | *Chukku* | *Zingiber officinale Rosc.* | Dried Rhizome | Gingerdione[21] |
| 7 | *Thippili* | *Piper longum L.* | Fruit | Piperlonguminine, (-)-beta-Bourbonene [22-23] |
| 8 | *Koduveli* | *Plumbago xeylanica L.* | Root | Plumbagin [24] |
| 9 | *Karunjeerakam* | *Nigella sativa L.* | Seed | Thymoquinone [25] |
| 10 | *Koduveli* | *Plumbago xeylanica L.* | Root bark | Plumbagin [24] |

**Table 2:Ligand Properties of the Compounds Selected for Docking Analysis**

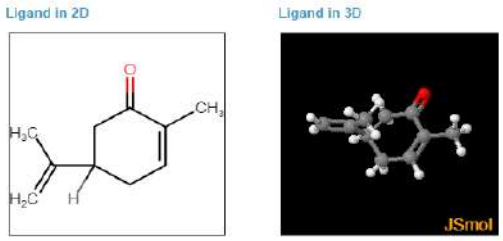
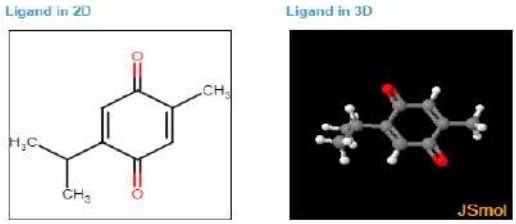
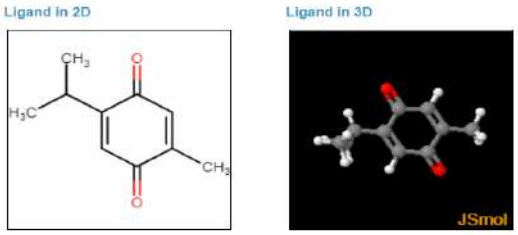
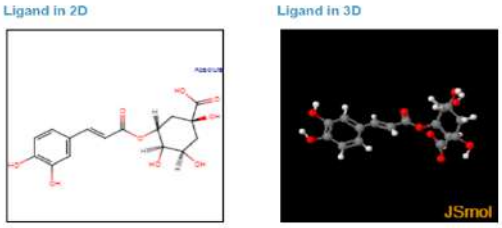
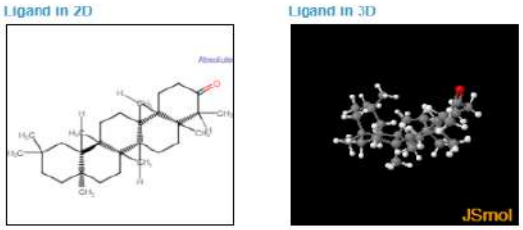
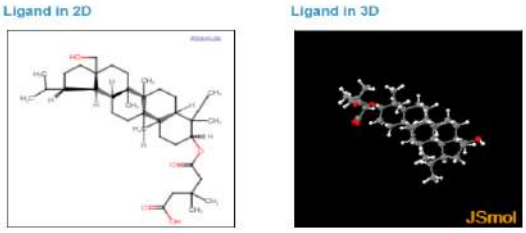
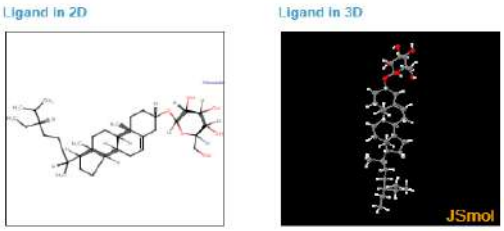
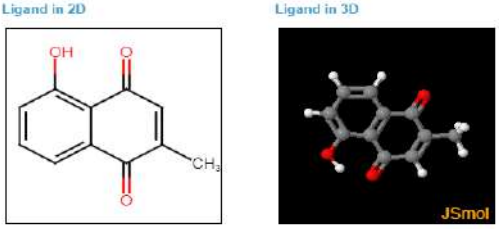
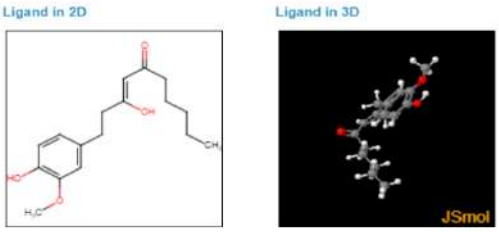
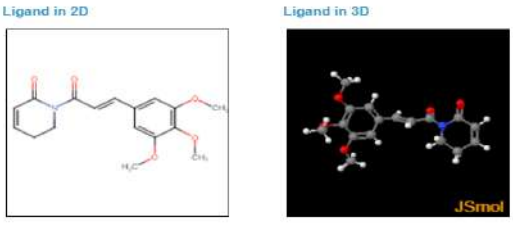
| Compound | Molar weight g/mol | Molecular Formula | H Bond Donor | H Bond Acceptor | Rotatable bonds |
|---|---|---|---|---|---|
| Carvone | 150.221 g/mol | $C_{10}H_{14}O$ | 0 | 1 | 1 |
| Thymoquinone | 164.204 g/mol | C10H12O2 | 0 | 2 | 1 |
| Cymene | 134.222 g/mol | C10H14 | 0 | 0 | 1 |
| Chlorogenic acid | 354.31 g/mol | $C_{16}H_{18}O_9$ | 6 | 9 | 5 |
| Friedelin | 426.7 g/mol | $C_{30}H_{50}O$ | 0 | 1 | 0 |
| Betulinic acid | 456.7 g/mol | $C_{30}H_{48}O_3$ | 2 | 3 | 2 |
| Daucosterol | 576.8 g/mol | $C_{35}H_{60}O_6$ | 4 | 6 | 9 |
| Plumbagin | 188.182 g/mol | $C_{11}H_8O_3$ | 1 | 3 | 0 |
| Gingerdione | 292.4 g/mol | $C_{17}H_{24}O_4$ | 1 | 4 | 10 |
| Piperlonguminine | 273.33 g/mol | $C_{16}H_{19}NO_3$ | 1 | 3 | 5 |
| Beta- Bourbonene | 204.35 g/mol | $C_{15}H_{24}$ | 0 | 0 | 1 |
| *Atorvastatin* | 558.6 g/mol | $C_{33}H_{35}FN_2O_5$ | 4 | 6 | 12 |

**Table 3: Summary of the molecular docking studies of compounds against HMG coA reductase (PDB) - 1HWK**

| Compounds | Est. Free Energy of Binding | Est. Inhibition Constant, Ki | Electrostatic Energy | Total Intermolec. Energy | Interact. Surface |
|---|---|---|---|---|---|
| Carvone | -4.70 kcal/mol | 356.83 uM | -0.01 kcal/mol | -5.00 kcal/mol | 476.564 |
| Thymoquinone | -4.86 kcal/mol | 276.10 uM | -0.02 kcal/mol | -5.15 kcal/mol | 491.272 |
| Cymene | -4.57 kcal/mol | 445.87 uM | -0.11 kcal/mol | -4.87 kcal/mol | 480.746 |
| Chlorogenic acid | -5.95 kcal/mol | 43.82 uM | -1.49 kcal/mol | -5.87 kcal/mol | 511.693 |
| Friedelin | -5.60 kcal/mol | 78.32 uM | -0.05 kcal/mol | -5.60 kcal/mol | 755.963 |
| Betulinic acid | -4.92 kcal/mol | 246.06 uM | -1.04 kcal/mol | -6.69 kcal/mol | 680.701 |
| Daucosterol | -5.88 kcal/mol | 49.32 uM | -0.01 kcal/mol | -7.38 kcal/mol | 861.588 |
| Plumbagin | -5.04 kcal/mol | 202.13 uM | -0.07 kcal/mol | -5.34 kcal/mol | 494.131 |
| Gingerdione | -3.86 kcal/mol | 1.48 mM | -0.06 kcal/mol | -5.74 kcal/mol | 708.618 |
| Piperlonguminine | -3.98 kcal/mol | 1.20 mM | -0.27 kcal/mol | -5.43 kcal/mol | 646.673 |
| Beta- Bourbonene | -6.03 kcal/mol | 38.10 uM | -0.06 kcal/mol | -6.33 kcal/mol | 558.994 |
| Atorvastatin | -5.55 kcal/mol | 85.94 uM | -1.30 kcal/mol | -6.17 kcal/mol | 648.525 |

**Table 4: Amino acid Residue Interaction of Lead against HMG coA reductase (PDB) - 1HWK**

| COMPOUND | INTERACTION | AMINO ACID RESIDUES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **CARVONE** | 0 | 655 MET | 766 GLN | 767 ASP | | | | | | |
| **THYMOQUINONE** | 2 | 691 LYS | 692 LYS | 693 PRO | 769 ALA | 772 VAL | | | | |
| **CYMENE** | 0 | 654 ALA | 655 MET | 766 GLN | 809 THR | | | | | |
| **CHLOROGENIC ACID** | 4 | 590 ARG | 682 ALA | 684 SER | 686 ASN | 690 ASP | 691 LYS | 692 LYS | | |
| **FRIEDELIN** | 4 | 590 ARG | 684 SER | 686 ASN | 690 ASP | 691 LYS | 692 LYS | | | |
| **BETULINIC ACID** | 3 | 590 ARG | 657 MET | 658 ASN | 661 SER | 683 VAL | 690 ASP | 691 LYS | 692 LYS | |
| **DAUCOSTEROL** | 3 | 590 ARG | 655 MET | 657 MET | 658 ASN | 683 VAL | 684 SER | 690 ASP | 691 LYS | 767 ASP |
| **PLUMBAGIN** | 2 | 691 LYS | 692 LYS | 769 ALA | 772 VAL | | | | | |
| **GINGERDIONE** | 4 | 590 ARG | 657 MET | 658 ASN | 661 SER | 684 SER | 686 ASN | 690 ASP | 691 LYS | 692 LYS |
| **PIPERLONGUMININE** | 4 | 590 ARG | 684 SER | 690 ASP | 691 LYS | 692 LYS | 769 ALA | | | |
| **BETA- BOURBONENE** | 0 | 655 MET | 766 GLN | 767 ASP | 809 THR | | | | | |
| **ATORVASTATIN** | 4 | 590 ARG | 657 MET | 658 ASN | 661 SER | 683 VAL | 684 VAL | 690 ASP | 691 LYS | 692 LYS | 767 ASP | 770 GLN |

| | |
|---|---|
|   |   |
| **Carvone** | **Thymoquinone** |
|   |   |
| **Cymene** | **Chlorogenic acid** |
|   |   |
| **Friedelin** | **Betulinic acid** |
|   |   |
| **Daucosterol** | **Plumbagin** |
|   |   |
| **Gingerdione** | **Piperlonguminine** |

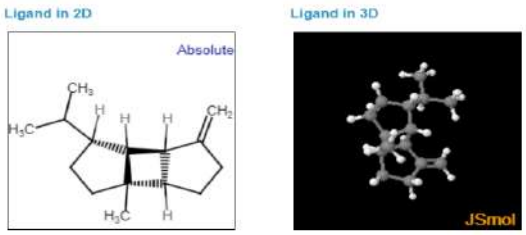
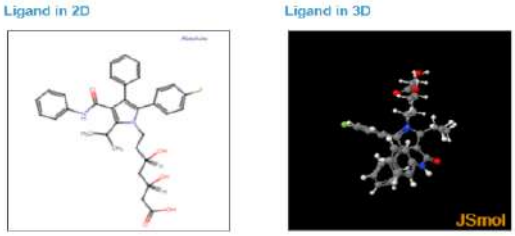
| | |
|---|---|
|   |   |
| **Beta- Bourbonene** | **Atorvastatin** |

**Fig: A. 2D & 3D Structure of Lead Compounds**

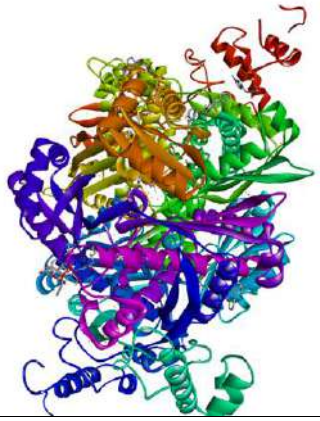
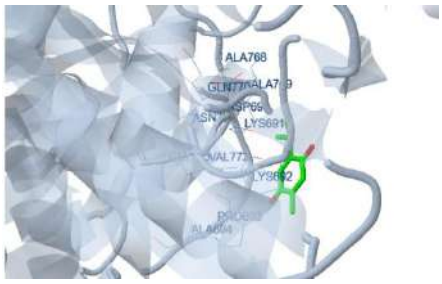
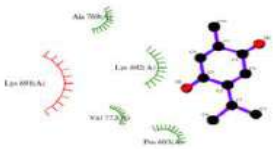
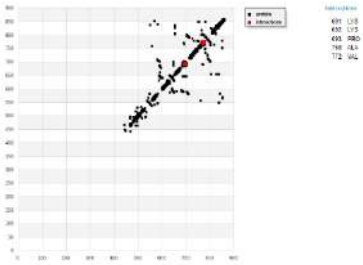
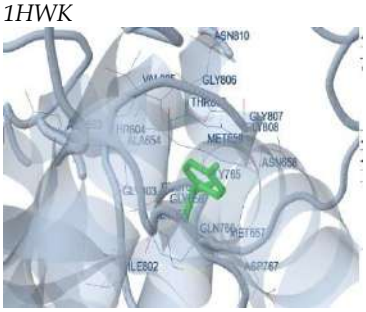
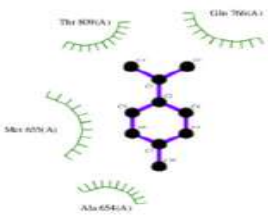
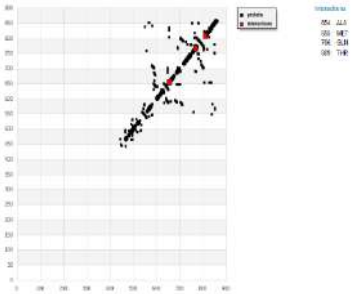


**Fig. B: 3D- Structure of HMG CoA reductase (PDB) - 1HWK**

| | | |
|---|---|---|
| *Thymoquinone with HMG CoA reductase (PDB) - 1HWK*  | *2D Interaction Plot Analysis*  | *Hydrogen bond plotting with core amino acid Analysis*  |
| *Cymene with HMG coA reductase (PDB) - 1HWK*  |  |  |

| | | |
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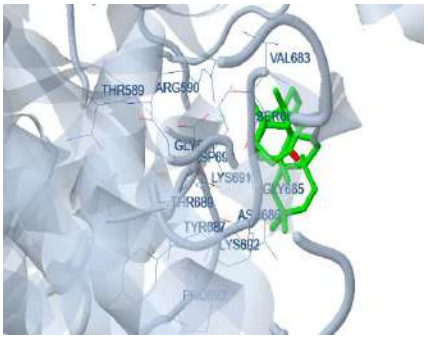
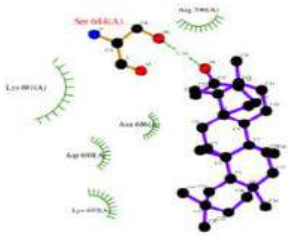
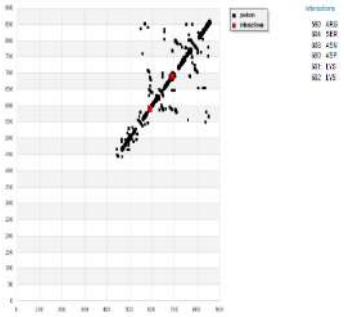
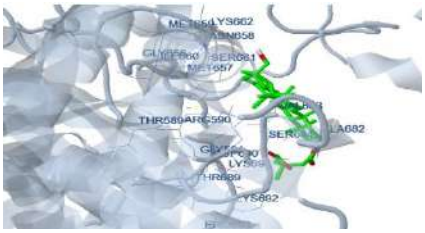
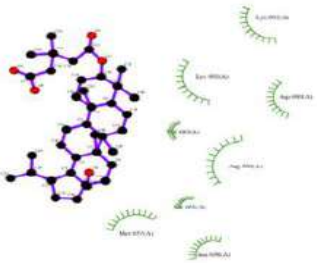
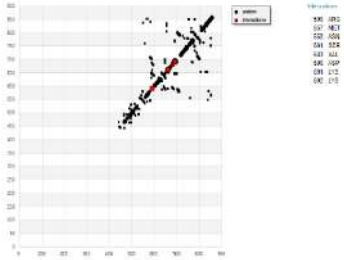
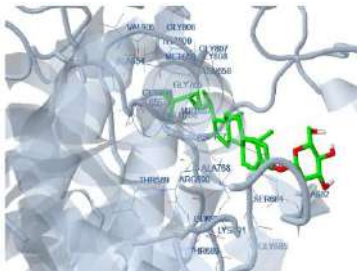
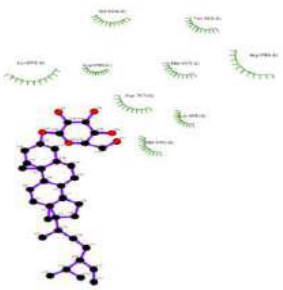
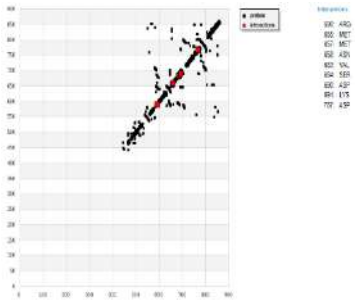
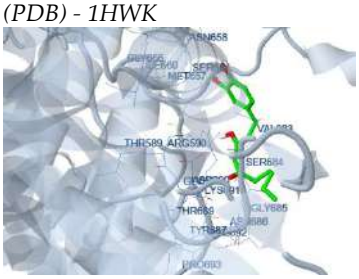
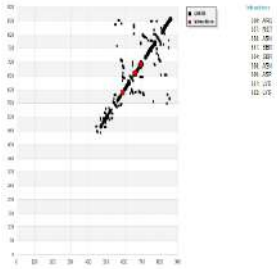
| *Friedelin with HMG coA reductase (PDB) - 1HWK*  |  |  |
| *Betulinic acid with HMG coA reductase (PDB) - 1HWK*  |  |  |
| *Daucosterol with HMG coA reductase (PDB) - 1HWK*  |  |  |
| *Gingerdione with HMG coA reductase (PDB) - 1HWK*  | |  |

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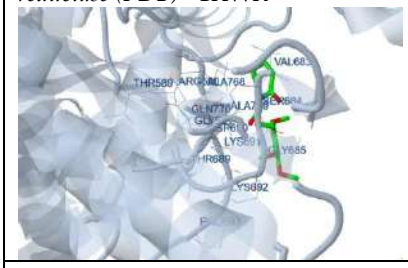
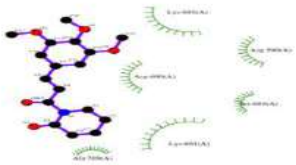
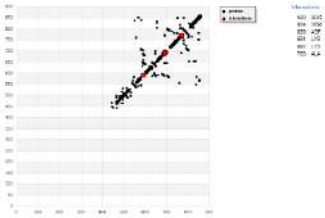
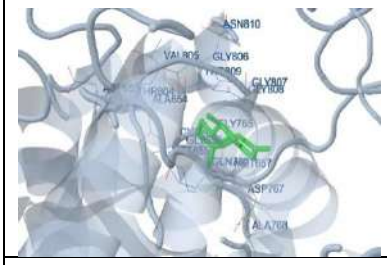
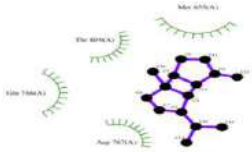
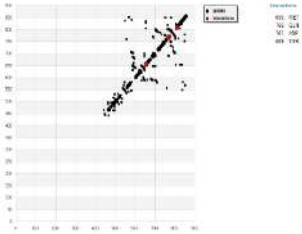
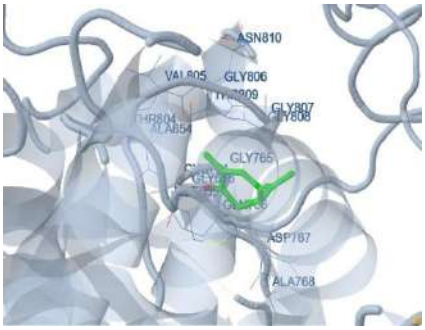
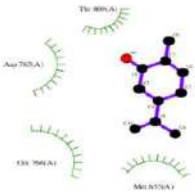
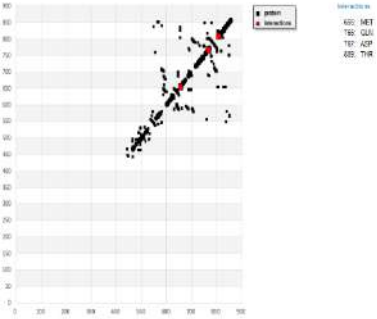
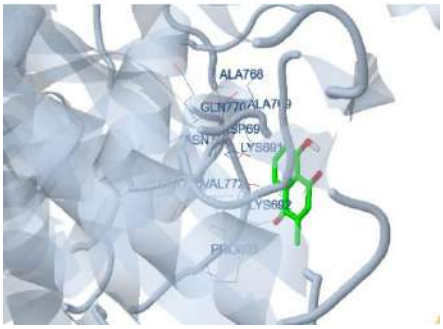
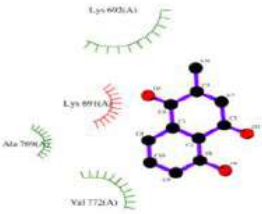
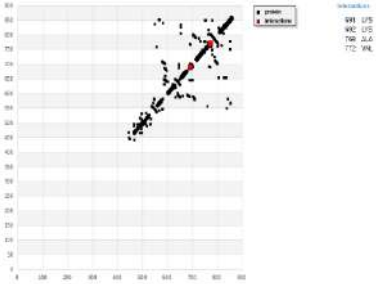
| *Piperlonguminine with HMG coA reductase (PDB) - 1HWK*  |  |  |
| *Beta- Bourbonene with HMG coA reductase (PDB) - 1HWK*  |  |  |
| *Carvone with HMG coA reductase (PDB) - 1HWK*  |  |  |
| *Plumbagin with HMG coA reductase (PDB) - 1HWK*  |  |  |

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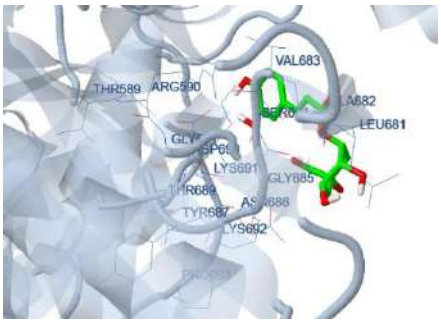
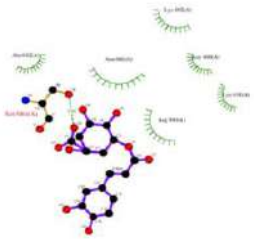
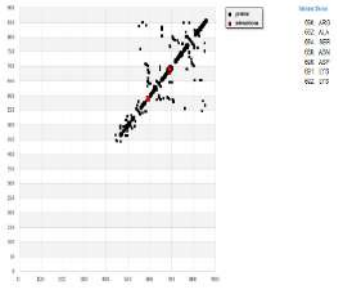
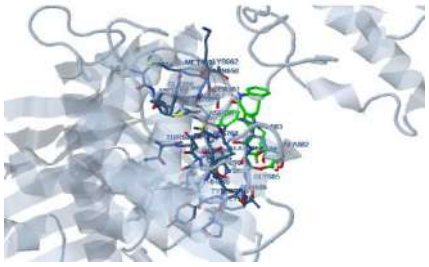
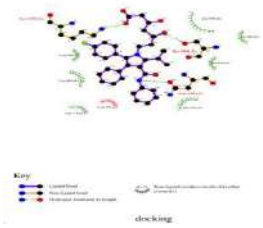
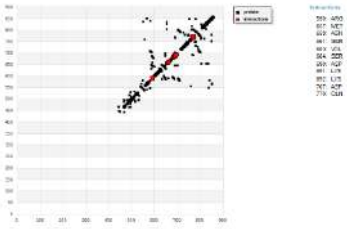
| *Chlorogenic acid with HMG coA reductase (PDB) - 1HWK*   |  |  |
| *Atorvastatin with HMG CoA reductase (PDB) - 1HWK*  |  |  |

**Fig. C. Amino acid interactions of lead molecules of SK & standard**

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**RESEARCH ARTICLE**

# Preliminary Phytochemical Screening and *In vitro* Antioxidant, Anti diabetic and Anti Mitotic Activity of Methanolic Extracts of *Hyophorbe lagenicaulis* Fruits

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**ABSTRACT**

The bottle palm, or *Hyophorbe lagenicaulis*, is a plant native to the Mascarene Islands that has traditionally been used in crafts and medicine. The pharmacological characteristics of its fruit extract are examined in this study, with an emphasis on the antimitotic, antidiabetic, and antioxidant effects. Bioactive substances such as phenols, tannins, saponins, steroids, triterpenoids, and carbohydrates were detected by preliminary phytochemical screening. The DPPH radical scavenging assay was used to assess the extract's antioxidant activity, and the results showed a dose-dependent inhibition with an IC50 value of 2.8 µg/mL. By assessing glucose absorption in a yeast cell model, the extract's antidiabetic efficacy was evaluated. At 10 mg/mL, it demonstrated an impressive 97.12% glucose absorption, outperforming the effects of the common medication metronidazole. By preventing seed germination in a green gram model, the extract also demonstrated antimitotic activity, indicating possible anticancer effects. These results demonstrate *Hyophorbe lagenicaulis*'s pharmacological potential as an antimitotic, antidiabetic, and antioxidant agent, bolstering its traditional medical usage and suggesting areas for future study.

**Keywords:** Bottle palmfruits, Anti oxidant, Anti diabetic, Anti mitotic, *invitro* screening.

## INTRODUCTION

The bottle palm, or *Hyophorbe lagenicaulis*, is native to the Mascarene Islands in the Indian Ocean, which are located east of Madagascar. Its native habitats are sandy, well-drained upland woodland and coastal savanna soils. It can thrive in the climates of the Hawaiian Islands, extreme southern California, and south Florida in the United States. Although it grows slowly, this tree can grow to a height of 12 to 20 feet. Although it may tolerate some shade, full sun is ideal for its growth. The 12-foot-long pinnately compound leaves or fronds are affixed to a 10-inch petiole or

stem. About two feet long, its dark green, thin, lance-shaped leaflets develop in a "V" pattern on the rachis, or center, of the frond, opposite one another. As it ages, the smooth, light grey to almost white trunk becomes more elongated and less apparent, but when it is young, its base is greatly increased. Above the stem, a smooth, waxy crownshaft, 2 to 3 feet tall, is where the leaf blades emerge. The trunk has massively branched flower inflorescences up to three feet long that surround it just below the crownshaft. Male and female flowers are seen on the same inflorescence and are either white or cream in color. The fruits are one inch in diameter and turn from green to black as they ripen [1].

**Varieties and Hybrids:**

1. *Hyophorbe lagenicaulis* 'Bottle Palm'
2. *Hyophorbe verschaffeltii* 'Spindle Palm'
3. *Hyophorbe amaricaulis* 'Hybrid Bottle Palm'

**Uses:**

1. Traditional medicine: using plants and roots to treat a variety of illnesses
2. Food source: Edible fruit that is not often eaten
3. Craft supplies: Leaves for thatching and weaving

**Phytochemistry:**

| Name | Phtyochemical | Pharmacological Activity |
|---|---|---|
| 1. Flavonoids: | Quercetin, Kaempferol | Antioxidant and anti-inflammatory effects [2]. |
| 2. Phenolic acids: | Ferulic acid, Sinapic acid | Antioxidant and antimicrobial properties [3]. |
| 3. Terpenoids: | Lupeol, Ursolic acid | Antimicrobial, antioxidant, and anti-inflammatory effects [3]. |
| 4. Alkaloids: | Hyophorine, Lagenine | Potential therapeutic applications [2]. |
| 5. Saponins: | Hyophorbesaponins | Antioxidant and anti-inflammatory effect [3]. |
| 6. Glycosides: | Flavonoid glycosides | Antioxidant and anti-inflammatory effects [2]. |
| 7. Phytosterols: | Stigmasterol, Sitosterol | Antioxidant and antimicrobial properties [3]. |

**Pharmacological Activities**

| Pharmacological activity | Mechanism of action |
|---|---|
| 1. Antimicrobial Activity | Antibacterial: effective against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [3]. |
| 2. Antioxidant Activity | Free radical scavenging: demonstrated potent antioxidant activity [3]. |
| 3. Anti-Inflammatory Activity | 1. Inhibition of inflammatory mediators: reduced inflammation in animal models [2].<br>2.Anti-inflammatory compounds: flavonoids, phenolic acids, and terpenoids[3]. |
| 4. Cytotoxic and Anticancer Activity | 1. Anti proliferative effects: inhibited cancer cell growth [3].<br>2. Apoptosis induction: triggered cell death in cancer cells [2]. |
| 5. Cardiovascular Protection | 1. Vasodilation: relaxed blood vessels, reducing blood pressure[3].<br>2. Antihypertensive effects: lowered blood pressure in animal models [2]. |
| 6. Neuroprotective Activity | 1. Neurotransmitter modulation: influenced neurotransmitter release [3].<br>2. Anticonvulsant effects: reduced seizure frequency and severity [2]. |
| 7. Hepatoprotective Activity | 1. Liver protection: prevented liver damage in animal models [3].<br>2. Antioxidant effects: reduced oxidative stress in liver cells [3]. |

## MATERIALS AND METHODS

**Materials:** Baker's yeast, Glucose, Methanol, Metronidazole, Vincristine sulphate Injection. All the chemicals used here are of analytical grade.

**Collection of plant material:** The fruits of bottle palm were collected from the premises of Vignan Institute of Pharmaceutical Technology, Visakhapatnam, Andhra Pradesh, India**.**

**Preparation of methanolic extract:** The collected plants were dried and made into powder. The powder was weighed and kept for maceration using methanol for 72 hours. The extract obtained was concentrated and stored in a refrigerator till further use.

**Preliminary Phytochemical Analysis:** The existence of phytochemicals like alkaloids, flavonoids, terpenoids, amino acids, cardiac and anthracene glycosides, saponins, steroids, tannins, phenols, and carbohydrates was confirmed by preliminary phytochemical analysis utilizing qualitative tests [5].

***invitro* Antidiabetic activity**

*invitro* Antidiabetic Activity Assessed by Glucose Uptake in a Yeast Cell Model The amount of glucose taken by yeast cells was measured using a unique technique created by Shettar et al[6]. Commercially available *Saccharomyces cerevisiae* (baker's yeast) cells were suspended in 1% solution and kept at 25° Celsius. To obtain the clear supernatant fluid, the yeast cell solution was centrifuged five times for five minutes at 4,200 rpm. The clear supernatant was used to make a 10% v/v yeast cell solution for the experiment. After mixing the mixture with 100 µL of yeast solution, the reaction was started. It was then vortexed and incubated for 60 minutes at 37°C.After that, the materials were centrifuged at 3,800 rpm for five minutes. Then, for five minutes, the materials was centrifuged at 3,800 rpm. at quantify the amount of glucose in the supernatant, a UV-vis spectrophotometer set at 520 nm was utilized. Every reagent is present in the control sample, with the exception of the test sample. The complete experiment was run in three repetitions.The percentage of glucose uptake was calculated by the formula:

$$\% \text{ of glucose uptake} = \frac{AbsControl\ -AbsSample}{AbsControl} \times 100$$

***In vitro* Antimitotic activity**

Preparing aqueous extracts and screening for antimitotic effectiveness using the *Vigna radiata* germination assay. Put a few green grams in a beaker with five milliliters of fresh water to mark them as the control. Five milliliters of fresh water should be added to a beaker along with a few green grams. Cover the beaker using filter paper. In another test tube, put a few green grams in a beaker having the extract solution. Cover the beaker using filter paper. It is best to keep both beakers at room temperature. Measure the length of the sprouts that grew in the test and control groups after three days. Compare the lengths of the test and control sprouts. Make a note of the observations [8].

## RESULTS AND DISCUSSION

**Preliminary Phytochemical Screening**

Preliminary Phytochemical analysis of bottle palm fruit extracts revealed the presence of carbohydrates, steroids and triterpenoids (Table 1).

***invitro* Antioxidant activity by DPPH Radical Scavenging Activity (DPPH)**

A dose-dependent inhibition was demonstrated by the extract in the dosage range of 2–10 mg/mL. The IC50 value is the amount of antioxidants in the sample required to 50% inhibit the free radicals. The IC50 value decreases as the radical scavenging ability increases. The extract showed dose-dependent activity with an IC50 value of 2.8 µg/ml and a maximum inhibition of 15.535% at 10 mg/mL. The positive standard ascorbic acid, on the other hand, showed an IC50 value of 12.8 µg/ml and 52.14% inhibition. The proportion of DPPH scavenge activity that *Hyphorbe lagenicaulis* inhibited was shown in the above graph [6] (Table 2).

***invitro* Antidiabetic activity by Glucose Uptake in Yeast Cell Model**

The glucose uptake test was used to evaluate in vitro antidiabetic activity of extracts from *Hyophorbe lagenicaulis* fruits at different dosages using a yeast model. Comparing the extract to the other extract doses, it demonstrated significant activity (97.12%) at 10 mg/ml.

As the amount of *Hyophorbe lagenicaulis* fruit extract raised from 2 to 10 mg/mL, the graph above demonstrates a dose-dependent rise in the percentage of glucose absorption. Metronidazole, a popular drug, demonstrated 70.5% at 10 mg/ml [7] (Table 3).

***invitro* Antimitotic activity by plant based models**

Green gram growth was observed in the control in water, however it was not observed in the test sample adding beaker. 0.5µg/ml of *Hyphorbe lagenicaulis* extract is the medication concentration used here. The standard chemotherapy drug Methotrexate, at 0.1 mg/ml, inhibited seed germination by 0.001±1.0 percent. Hence, the extract has strong anti-mitotic properties [8].

## CONCLUSION

The phytochemicals found in the plant extract *Hyphorbe lagenicaulis*, which is the subject of the preliminary phytochemical screening; include phenols, tannins, saponins, steroids, triterpenoids, and carbohydrates. As part of the current study, the fruit extract of *Hyphorbe lagenicaulis* was tested for antioxidant potential. The DPPH method was regarded as a stable technique since its outcomes are independent of the substrate's polarity. The primary idea underlying the active ingredients in plant extracts scavenging free radicals may be that they provide hydrogen to the free radicals, neutralising them and generating stable compounds that lessen their deadly consequences. With a maximum DPPH scavenging activity of 15.535%, the extract's IC50 was 2.8 mg/mL, while the standard IC50 was 12.8 mg/mL. Using yeast cells and the uptake of glucose technique, the antidiabetic efficacy of *Hyophorbe lagenicaulis* was evaluated in vitro. One significant indicator of yeast cells' uptake of glucose was the quantity of glucose left in the solution after a predetermined period of time. The percentage of glucose absorption by yeast cells mediated by plant extracts is 97.12% at 10 mg/ml, while the percentage of metronidazole uptake is 70.5% at 10 mg/ml, demonstrating the *invitro* antidiabetic action of *Hyophorbe lagenicaulis* extract using a yeast cell model. With the help of stereospecific membrane carriers, the diffusion mechanism facilitated the movement of glucose across the yeast cells' cell membrane. *Hyophorbe lagenicaulis* methanol extract demonstrated improved glucose utilisation, which increases glucose uptake efficiency and helps regulate blood glucose levels. According to this study, *Hyophorbe lagenicaulis* has the potential to have antimitotic effects. The extract has demonstrated antimitotic action and seed germination inhibition at a dosage of 0.5 mg/ml.

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**Table 1: Phytochemicals present in Bottle palm fruits**

| S.No | Name of the test | Observation | Inference |
|---|---|---|---|
| 1. | **Alkaloids**<br>Wagner's test | No reddish color ppt is formed. | **Alkaloids are absent** |
| 2. | **Amino acids and Proteins**<br>Ninhydrin test:<br>Millon's test: | No development of purple or bluish colour.<br>No development of brick red colour. | **Amino acids and proteins are absent** |
| 3. | **Carbohydrates**<br>Molisch's test:<br>Fehling's test: | Purple ring at the junction of 2 layers.<br>Presence of brick red colour. | **Carbohydrates are present** |
| 4. | **Glycosides**<br>Legal test: | No pink or red color obtained. | **Glycosides are absent** |
| 5. | **Flavonoids**<br>Shinoda test: | No yellow ppt is formed and no pink color is observed. | **Flavonoids are absent** |
| 6. | **Tannins**<br>Ferric chloride test: | Deep blue-black color is obtained. | **Tannins are present** |
| 7. | **Saponins**<br>Froth formation test: | Persistent foam is observed. | **Saponins are present** |
| 8. | **Phenols**<br>Lead acetate test: | Presence of white ppt. | **Phenols are present** |
| 9. | **Steroids and Triterpenoids**<br>Salkoswski test | Reddish blue color in chloroform layer. | **Steroids and Triterpenoids are present** |

**Table 2: *invitro* Antioxidant Activity of Bottle palm fruits**

| Concentration | Sample Absorbance | % inhibition of DPPH Radical | Absorbance of standard ascorbic acid | %inhibition of DPPH radical of standard ascorbic acid |
|---|---|---|---|---|
| 2mg/ml | 0.382 | 8.49% | 0.325 | 22.23% |
| 6mg/ml | 0.369 | 11.0% | 0.230 | 36.96% |
| 10mg/ml | 0.353 | 15.535% | 0.200 | 52.14% |

**Fig.1. Fruits**

| Absorbance control=0.1450 | | | | |
|---|---|---|---|---|
| Concentration | Sample absorbance | %glucose uptake | Absorbance of Metronidazole | % Glucose uptake of Metronidazole |
| 2mg/ml | 0.0657 | 54.68% | 0.0320 | 38% |
| 4mg/ml | 0.0404 | 74.44% | 0.0280 | 46% |
| 6mg/ml | 0.0445 | 69.31% | 0.0230 | 55% |
| 8mg/ml | 0.0199 | 86.27% | 0.0519 | 62% |
| 10mg/ml | 0.0041 | 97.12% | 0.0153 | 70.5% |

***Fig 2. invitro* Antioxidant activity by DPPH Radical Scavenging Activity**

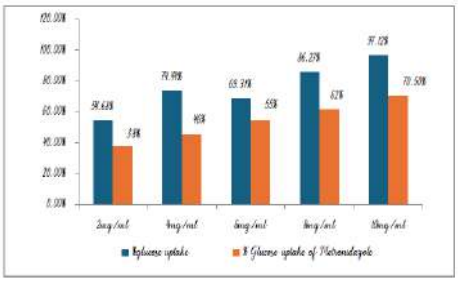


***Fig 3.In vitro* Antidiabetic activity by Glucose Uptake in Yeast Cell**

**Figure 4. Green gram Models**

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**RESEARCH ARTICLE**

# Preliminary Phytochemical Screening and *Invitro* Pharmacological activities of *Hymenocallis littoralis*

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**ABSTRACT**

Originally from theLatin American coastal locations that are warmer, *Hymenocallis littoralis*, also referred Spider lily is a type of plant belonging to the Amaryllidaceae family that has spread throughout tropical regions across the world. The pharmacological potential of the plant is examined in this study, with particular attention to its antimitotic and antidiabetic properties. A yeast cell glucose uptake model was used to evaluate the methanol extract of *H. littoralis's* antidiabetic properties in vitro. The extract outperformed the reference medication metronidazole, which exhibited 70.5% absorption at the same concentration, with an 83.4% glucose uptake at 10 mg/mL. The findings demonstrate the extract's capacity to improve glucose utilisation and successfully control blood glucose levels by showing that glucose transport across yeast cell membranes was made possible by stereospecific membrane carriers. Furthermore, *H. littoralis* showed strong antimitotic action, as evidenced by its capacity to prevent seed germination at 0.5 mg/mL. This implies the existence of bioactive substances that have a variety of physiological and medicinal impacts, especially when it comes to the treatment of cancer. The results highlight *H. littoralis's* potential as a natural chemical source for creating anti-cancer and antidiabetic treatments, calling for more research into its bioactive components and modes of action.

**Keywords:** *In vitro screening,* Anti diabetic, Anti mitotic, Aerial parts, Spider lilly

## INTRODUCTION

Spider plants the Amaryllidaceae family comprises Hymenocallis littoralis., is the botanical name for lily. Originally from warmer coastal regions of Latin America, this plant species belongs to the genus *Hymenocallis* and is now widely grown and naturalised in many tropical nations [1] .The Beach Spider Lily has narrow, sword-shaped leaves

and grows at a height of 30 to 70 cm. The beach spider lily plant produces two to twelve flowers in a cluster, and a long stalk that is about two feet long emerges from the middle of the leaves. The flower is called Spider Lily because its petals resemble the legs of a spider. It's a significant medicinal herb. Certain plant parts, including leaves, have antibacterial and anti-inflammatory qualities [2]. Only the bulb of the plant is used to cure wounds; the extract from the roots and rhizomes is used as a nasal drop for snake bites. Numerous alkaloids have been identified from the spider lily plant's bulb during the course of its history. Several plants in the Amaryllidaceae family have been identified as having alkaloids, which are known to have a broad range of pharmacological activities [3]. Numerous alkaloids, including homolycorine, lycoramine, vittatine, macronine, lycorenine, tazettine, pretazettine, hippeastrine, littoraline, lycorine, and haemanthamine had been extracted from spider lily plants. Numerous pharmacological actions, including antibacterial, antiparasitic, antiviral, anticancer, antioxidant, and wound-healing characteristics,have been documented for these substances [4,5]. One of the main alkaloids in *Hymenocallis littoralis* [6] is lycorin, a pyrrolophenanthridine alkaloid.

**Phytochemistry**

| S.No | Part | Compound |
|---|---|---|
| 1 | Bulbs | Alkaloids include (þ)-8-O-demethylmaritidine6, lycorine (1), hippeastrine (2), and 11-hydroxyvittatine (3). |
| 2 | Flowers | Rutin and quercetin 3'-O-glucoside (5) are flavonoids.<br>Citronellal, hydroxycitronellal [7], along with other volatile oils |

**Pharmacological Studies**

•**Methylflavan /Antioxidant:** 7,4'-dihydroxy8-methylflavan was extracted from the *H littoralis*stem extract and evaluated for its capacity to scavenge radicals [8].
•**Cytotoxicity:** Pancratistatin (PST), which was derived from *H littoralis* in 1993, demonstrated substantial cytotoxicity to a human cancerous cell line. PST is selective for cancer cells while sparing healthy cells unaltered, per a new study. In this work, two natural compounds associated with PST, AMD4 and AMD5, were evaluated for their anti-cancer activity and specificity. The findings demonstrated that AMD4 lacked apoptotic activity while AMD5 exhibited selectivity and efficacy comparable to PST. Natural Amaryllidaceae alkaloids may share a phenanthridone structure that contributes to their specificity in combating cancer [9].
•**Anti-tumor:**In vitro and in vivo, the biologic activities of isocarbostyril alkaloids revealed greater cytotoxicity against a variety of tumour cell lines, together with strong selectivity for cancer cells relative to normal cells.Ten. Littoraline, HI Reverse Transcriptase Inhibition, Lycorine Alkaloids, and Cytotoxicity: The study uncovered a novel alkaloid, littoraline, in addition to 13 other known lycorine alkaloids and one lignan. In vitro, littoraline showed HIV reverse transcriptase inhibitory activity, but lycorine and haemanthamine showed high cytotoxicity [10].
•**Lycorine Alkaloids / Littoraline / HI Reverse Transcriptase Inhibition / Cytotoxicity:** Along with 13 other known lycorine alkaloids and one lignan, the study isolated a novel alkaloid, littoraline. Lycorine and haemanthamine demonstrated strong in vitro cytotoxicity, while littoraline demonstrated HIV reverse transcriptase inhibitory action.
•**Pancratistatin/Anticancer:** (1) The species is a good source of the potent anticancer drug pancratistatin. The bulbs produce the majority of pancratistatin, with the roots producing a smaller amount. A large-scale production method is described in the study. (2) Narciclasine was used as a precursor to create natural (+) pancratistatin synthetically.
•**Alkaloids:**Phytochemical examination of bulbs and flowers demonstrated the presence of four alkaloids, including lycorine, hippeastrine, 11-hydroxyvittatine, and (+)-8-O-demethylmaritidine, along with two flavonoids, quercetin, 3-O-glucoside, and rutin. In a petroleum ether extract [11], the antibacterial qualities of the flowers were analysed.
•**Narcistatin/Antiteoplastic:**Antecedents of the human cancer cell line inhibitory isocarbostyril have been obtained from the bulbs of *Hymenocallis littoralis* [12], which is derived from the horticultural production or reduction of narciclasine 1a-4 from the same source .
•**Anti-Candida Activity:** A methanol extract of several plant components was tested for its ability to inhibit Candia albicans. At 6.25 mg/ml [13], the flower and anther had an impact.

•**Antimicrobial:** An aqueous extract was tested against Candida albicans, S. aureus, and E. coli. All of the examined species were inhibited by varying concentrations.

•**Antibacterial:** Leaf, flower, and stem bark extracts in methanol and ethyl acetate shown antibacterial action against *B.subtilis* [14]. The bulbs are frequently used as decorative plants and in the manufacturing of cosmetics.

## MATERIALS AND METHODS

**Materials:** Vincristine sulphate injection, glucose, methanol, metronidazole, and baker's yeast. Here, only analytical-grade compounds are utilised.

**Collection of plant material**: In Visakhapatnam, Andhra Pradesh, India, the Vignan Institute of Pharmaceutical Technology is where the entire Spider Lilly plant was gathered.

**Preparationof methanolic extract:** involved drying and powdering the gathered botanicals. The powder was weighed and allowed to macerate for seventy-two hours in methanol. The resulting extract was concentrated and refrigerated until it was needed again.

**Preliminary Phytochemical Analysis:**Using qualitative tests, a preliminary phytochemical study was carried out to validate the presence of various phytochemicals, such as alkaloids,polysaccharides, terpenoids, flavonoids, anthracene and cardiac glycosides, saponins, steroids, tannins, phenols

***in vitro* Antidiabetic activity**

Analysing Glucose Uptake in a Yeast Cell Model to Assess Antidiabetic Activity in vitro The special method developed by Shettar et al. was used to measure the amount of glucose absorbed by yeast cells.. At 25°C, aA 1% solution of commercially available yeast cells, Saccharomyces cerevisiae, was added. To get the clear supernatant fluid,Five minutes of repeated centrifugation at 4,200 rpm was carried out on the overnight yeast cell solution.For the experiment, a 10% v/v yeast cell solution was produced with the clear supernatantThe mixture was mixed with 100 µL of yeast suspension, and the reaction was initiated. At 37°C, it was vortexed and incubated for 60 minutes. Except for the tAfter that, the materials were centrifuged at 3,800 rpm for five minutes. Using a UV-vis spectrophotometer set at 520 nm, the amount of glucose in the supernatant was estimated.The control sample contains every reagent except for the test sample. The experiment was carried out three times in duplicate. To calculate the percentage of glucose uptake, the following formula was applied:

$$\% \text{ of glucose uptake} = \frac{AbsControl\ -AbsSample}{AbsControl} \times 100$$

***In vitro* Antimitotic activity**

Weighed individually, green gram seeds were collected from the local grocer. 10 mg/ml, 20 mg/ml, 30 mg/ml, and 40 mg/ml extract concentrations were created. In this case, methotrexate is the standard. The control that we used was distilled water. We added equal weights of seeds to the mixture in Petri plates with different concentrations. A full day was spent at room temperature with the Petri plates in order to imbibition water. The pharmacological treatment was dried on dry tissue paper and weighed after 24 and 72 hours. We extended the sprouting period to 72 hours and took images.

where the seed's weight in distilled water is equal to Wt D.

The extract sample's seed weight is equal to (Wt E).

Seed weight in methotrexate = (Wt M)

## RESULTS AND DISCUSSION

**Preliminary Phytochemical Analysis**

Alkaloids, amino acids, carbohydrates were among the phytochemicals present in *Hymenocalis* (Table 1).

**Anti diabetic by glucose uptake method**

Using a yeast model, the glucose uptake test was used to evaluate the in vitro antidiabetic efficacy of *H. littoralis* extract at varying doses. At 10 mg/mL, the extract showed strong activity (83.4%) in comparison to the other extract doses. The graph above showed a dose-dependent increase in the percentage of glucose absorption as the concentration of H. littoralis extract increased from 2 to 10 mg/mL. At 10 mg/mL, the common medication metronidazole showed 70.5%.

**Anti mitotic methods by plant based models**

The laboratory results demonstrated that the green gram methanolic extract considerably raised the percentage inhibition following treatments of 24, 48, and 72 hours, which was comparable to the percentage inhibition of methotrexate. A 40 mg/ml concentration of methanolic extract resulted in 0.01±0.1 mm of seed growth, meaning that a 0.1 mg/ml concentration of the common medication Methotrexate inhibited seed germination by 0.001±1.0. After 24 hours, 48 hours, and 72 hours of treatment, the assay findings demonstrated that the methanolic extract of green grams greatly reduced the percentage of inhibition, which was comparable to the percentage of inhibition of methotrexate. The methanolic extract with a 20 mg/ml concentration demonstrated 0.98±0.9 mm seed growth. When considering seed germination, distilled water, the control, has demonstrated more seed germination than standard and methanolic extracts (Table 3).

## CONCLUSION

Yeast cell absorption of glucose, a profitable metric to evaluate the antidiabetic activity under *in vitro* settings, was used to test the antidiabetic activity of *H. littoralis*. The amount of glucose that was still present in the medium after a predetermined period of time was one prominent indicator of the uptake of glucose by yeast cells [16]. In a yeast cell model, the extract's in vitro antidiabetic effect can be determined by the fact that the percentage of glucose absorption by yeast cells mediated by *H. littoralis* plant extracts is 83.4% at 10mg/ml, while the percentage of Metronidazole uptake is 70.5% at 10 mg/ml.

Diffusion enables glucose to pass across the yeast cell membrane with the help of stereospecific membrane carriers. This demonstrated unequivocally that the methanol extract of *H. littoralis* is more effective at absorbing glucose by improving its utilisation, which in turn helps regulate blood glucose levels. According to this investigation of *H. littoralis's* antimitotic activity, the plant may have antimitotic properties. This action demonstrated the existence of numerous important chemical compounds that are in charge of producing different physiological and medicinal effects. Therefore, this plant may be utilised as a source of cancer treatment medications. The extract has demonstrated antimitotic action and seed germination inhibition at a dosage of 0.5 mg/ml. According to this study, *H.littoralis's* may possess antimitotic qualities. This plant might therefore yield medications that could be used to treat cancer. Following treatment for 24, 48, and 72 hours, the methanolic extract of green grams showed a significantly larger percentage inhibition than methotrexate.The antimitotic activity of a 40 mg/ml methanolic extract was 0.01±0.1 mm, which indicates that the percentage suppression of seed germination was comparable to that of the common drug Methotrexate, which is 0.001 mg/ml±1.0 mm.

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**Table 1. Prelimnary Phytochemical analysis**

| S.No | NAME OF THE TEST | Observation | Inference |
|---|---|---|---|
| **1** | **Alkaloids**<br>Dragendroff's test<br>Wagner's test | There was an orange-brown precipitate visible.<br>There was a reddish-brown precipitation. | Alkaloids are present. |
| 2. | **Amino acids**<br>Ninhydrin test | Purple color was observed. | Amino acids are present. |
| 3. | **Carbohydrates**<br>Molisch's test | At the intersection, a purple ring develops. | Carbohydrates may present. |
| 4. | **Glycosides**<br>Borntrager's test | No change of ammonia layer. | Anthraquinone glycosides are absent. |
| 5. | **Flavonoids**<br>Shinoda test | No pink colour observed. | Flavonoids are absent. |
| 6. | **Tannins**<br>Ferric chloride test | There was no blue-black precipitate visible. | Tannins are absent. |
| 7. | **Saponins**<br>Froth formation test | No persistant observed. | Saponins are absent. |
| 8. | **Steroids and Triterpenoids**<br>Liebermann Burchards test | No colour change was observed. | Steroids are absent. |

**Table 2. *invitro* Antidiabetic activity**

| Absorbance control = 0.145 | | |
|---|---|---|
| **Concentration** | **Sample absorbance** | **% Glucose uptake** |
| 2mg/ml | 0.0994 | 31.44% |
| 4mg/ml | 0.0802 | 44.6% |
| 6mg/ml | 0.0623 | 57.03% |
| 8mg/ml | 0.0363 | 74.9% |
| 10mg/ml | 0.0240 | 83.4% |

**Table 3. *invitro* Antimitotic activity**

| S.No | Drug Treatment | Concentration mg/ml | Treatment schedule | Seed growth's average |
|---|---|---|---|---|
| 1 | Methanolic extract-1 | 10 | 24 to 72 hours | 1.8±4.8 |
| 2 | Methanolic extract-2 | 20 | 24 to 72 hours | 0.98±0.9 |
| 3 | Methanolic extract-3 | 30 | 24 to 72 hours | 0.55±0.6 |
| 4 | Methanolic extract-4 | 40 | 24 to 72 hours | 0.01±0.1 |
| 5 | Control (Distilled water) | 0 | 24 to 72 hours | 29.5±5 |
| 6 | Standard – Methotrexate | 0.1 | 24 to 72 hours | 0.001±1.0 |

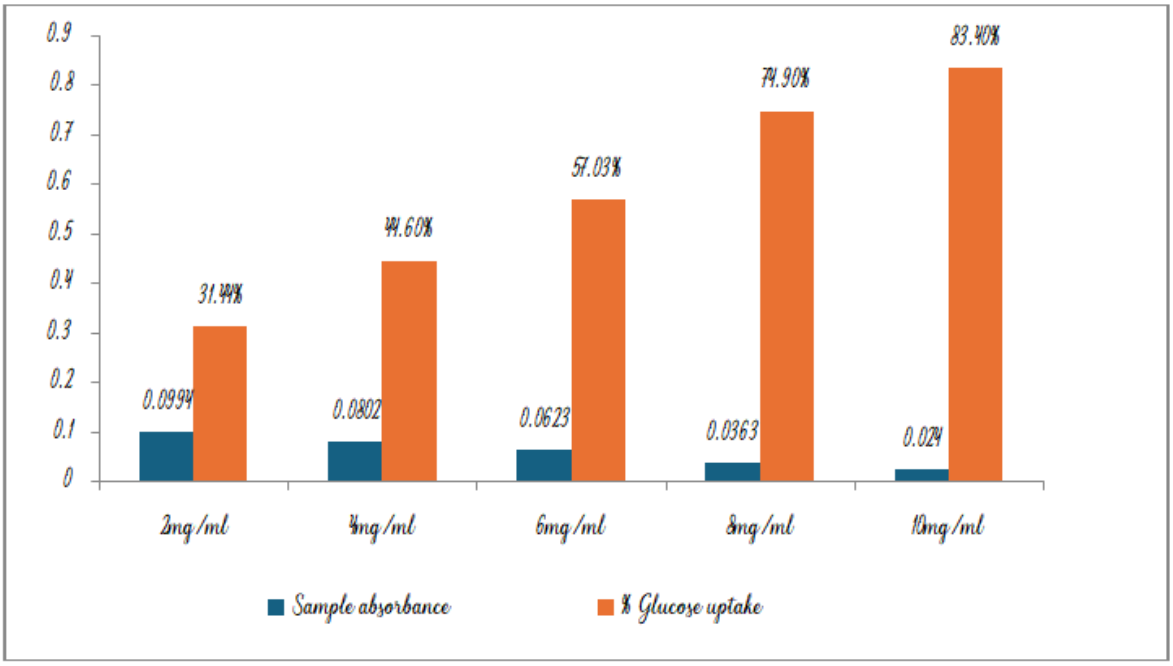


**Figure 1 *invitro* Antidiabetic activity**

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**RESEARCH ARTICLE**

# Development and Characterization of Tooth paste Containing Casein Microspheres

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## ABSTRACT

The aim of present research to develop toothpaste containing aloevera and casein. Aloe gel has anti-bacterial and anti fungal properties & it has good binding properties. This is used as a binding agent and therapeutic agent in development of toothpaste.Casein has anti- tooth decaying property, So Casein is incorporated as microspheres. These microspheres are added to the prepared toothpaste. Toothpaste was formulated by using a different proportions of various components. The toothpaste prepared which can accept all the required condition to keep the mouth feel fresh and prevent tooth decay. The prepared toothpaste was evaluated for different parameters such as homogeneity, spreadability, foaming ability,stability, PH, moisture and volatile material and etc. All these tested parameters were compared with marketed formulations. It has a good scope in future dental health of public.

**Keywords:** Casein,aloevera,Tooth decay,Foaming ability

## INTRODUCTION

One of the most popular preventative measures for oral health is toothpaste. Although several commercial dentifrices make claims regarding their antibacterial qualities, little study has been done to support these claims. The purpose of this study was to evaluate how well different toothpaste formulations reduced the levels of oral bacteria. Improved oral hygiene was facilitated by the evaluated formulations' capacity to regulate the microbial burden. However, sustaining oral health still depends more on good brushing habits and oral hygiene habits than on the particular components of toothpastes[1] Toothpaste is a paste formulation product and is used to clean and maintain oral hygiene with the aid of toothbrush [2]. In order to avoid dental problems like cavities, gingivitis, and periodontal disorders, maintaining oral hygiene entails keeping the mouth and teeth clean. Oral infections typically result from the activity of plaque-forming bacteria and yeast present in the oral cavity, including species like *Actinomyces*, *Actinobacillus*, *Streptococcus*, and *Candida [3].*

Plaque-forming bacteria and yeast, such as Streptococcus, Actinomyces, Actinobacillus, and Candida, are commonly found in the oral cavity and are responsible for oral infections. The phosphoprotein casein is involved in controlling the body's metabolism of calcium and phosphate. Improved bone mineralisation has been shown in experimental animals, especially in postmenopausal models. By increasing the calcium phosphate content of dental plaque, casein proteins can also aid in the prevention of tooth decay. Variants of casein, including $\alpha$-casein, β-casein, $\alpha$s1-casein, $\alpha$s2-casein, and κ-casein, can be transformed into bioactive molecules. For instance, $\alpha$-casein can produce casomorphine, an opioid peptide, while $\alpha$- and β-caseins act as precursors to immunopeptides[4]. The uses of aloe vera gel, a natural and age-old substance, in dentistry have been well investigated. It has shown promise in the treatment of oral mucosal lesions, gingivitis, wound healing, and plaque reduction[5].

## MATERIALS AND METHODS

**Extraction of aloevera gel**

The fresh Aloe barbedensis miller leaves were collected and washed with water. Incisions were made on the leaves and left over night.The gel was scraped off from the pods of aloevera. The Aloe gel was extracted using a multilayer muslin cloth bag to remove the marc from the solution and extraction process was shown in the Figure-1.[6,7,8]

**Toothpaste formulation**

The required quantity of ingredients were weighed according to the formulation table1 and prepared four different formulations based on different proportions of carboxymethyl cellulose and aloevera gel. The Calcium di-phosphate , Sodium Lauryl sulfate (S.L.S),and carboxymethyl cellulose were macerated in mortar while silica gel, propylene glycol. sorbitol and sodium saccharine were dissolved in water. The macerated powders were mixed with the prepared Aloevera gel solution.

**Preparation of Casein Microspheres**

0.8g of casein and 1g of sodium alginate were weighed accurately and taken into a clean and dry mortar and colouring agent was added. 10 ml of distilled water was added to the above contents and triturated well by adding small quantities of water until the desired consistency was acquired. The mixture was kept aside for 15 min. Now the mixture was taken into the syringe (syringe containing 18 gauze needle) and slowly released the mixture drop wise into the 10% $CaCl_2$ solution, left undisturbed for 20 minutes and filtered the solution and collected the beads. The beads were dried by keeping in hot air oven at 70 C for about 12 hours. After drying the rigid microbeads was obtained. Collected the dried beads and the evaluation tests were performed.The preparation process was shown in Figure-2.

**Addition of Casein Microspheres to Toothpaste**
Prepared Casein Microspheres were added to the formulated toothpaste and mixed gently until uniform distribution of microspheres in the tooth paste.

**Evaluation of tooth paste [**9,10,11**] :**
All the four formulations of tooth paste were evaluated for various parameters

**Physical Examination (Colour, Odour, Taste, Smoothness)**
Formulated tooth paste was evaluated for its colour.
The visually colour was checked.
Odour was found by smelling the product.
Taste was checked manually by tasting the formulation.
The Smoothness was tested by rubbing the formulation between the fingers.

**Relative density**
Relative density was determined by weight in gram taken in 10 ml formulation and 10 ml distilled water using specific gravity bottle.

**Transparency**
About 5 millilitres of the prepared paste were placed in a 10-milliliter test tube, and the transparency of the mixture was visually examined.

**Homogeneity**
Using normal force at 27±20C, the toothpaste should extrude a homogenous mass from the collapsable tube or any other appropriate container. Additionally, the majority of the contents must protrude from the container's crimp before it is gradually rolled.

**Determination of sharp and edge abrasive particles**
On the butter paper, extrude the contents that are 15 to 20 cm long. Do this for ten or more collapsable tubes. Check for the presence of hard-edged, sharp abrasive particles by pressing the contents of the entire length with the tip of your finger. Such particles must not be present in tooth paste.

**Microbial Growth**
This technique made use of nutritional agar medium. The prepared paste sample was aseptically transferred in a cross pattern onto the sample plate using the blank and sample petriplates. Microbial growth was monitored constantly for up to 15 days.

**Foaming Ability (6)**
V2 is the volume of water alone in millilitres, and this is the volume of foam with water in millilitres. Examined is the toothpaste's capacity to create foam during brushing, which enhances the brushing experience overall. A tiny quantity was combined with water in a measuring cylinder, and the initial volume was noted in order to evaluate the toothpaste's foamability. After shaking the mixture 10 times, the final volume of foam was noted. The froth power was calculated using the formula: Foaming power = V1−V2, where V1

**pH**
A pH meter was used to measure the formed paste's pH. This process involved dispersing 1 g of paste in 100 ml of filtered water. Prior to use, the electrode was calibrated using a standard buffer solution at 4.0, 7.0, and 9.0 after being cleaned with double-distilled water and dried with tissue paper. Three separate pH readings were taken, and the averages were computed.

**Estimation of moisture and volatile content**

5 g of paste placed in a porcelain dish having 6-8 cm in diameter and 2-4 cm depth in it. Dry the sample in oven at $105^0$C.

**Calculation**

% by mass=100MI/M

MI- Loss of mass(g) on drying

M- Mass (g) of the material taken for the test.

**Viscosity**

It was determined by using viscometer (Brookfield) with 2 number spindles.

**Extrudability**

This process involved filling a conventional capped collapsable laminated tube with the prepared paste, then crimping the end to seal it. The tubes' weights were noted down. The tubes were clamped after being positioned between two glass slides. After covering the slides with 500 g, the cap was taken off. Weighing was done on the quantity of extruded paste. It was calculated as a percentage of the extruded paste.

**Spreadability**

This technique uses the paste's slip and drag properties. 2g of the prepared paste was applied to the ground slide being examined. To release air and create a consistent layer of paste between slides, the prepared paste was sandwiched between this slide and another glass slide for five minutes. From the edges, extra gel was scraped off. The time (Sec) needed for the top slide to travel 7.5 cm was then recorded when the top plate was pulled 80 g with the aid of rope fastened to the hook. Better spreadability was indicated by a short interval.

Formula was used to calculate spreadability:

$S=M \times L/T$

Where,

S= Spreadability

M= Weight in the pan (tied to the upper slide) L= Length moved by the glass slide

T= Time (Sec) taken to separate the upper slide from the ground slide.

**Storage stability:**

The toothpaste was placed in a toothpaste tube and kept at 50 degrees Celsius, room temperature, and 400 degrees Celsius for 45 days. After cutting through the tube, the following criteria were used to determine whether or not the liquid component had been separated from the toothpaste. Storage stability evaluation criteria:

A) No liquid component separation is seen.

B) There is a small separation of a liquid component.

C) It is evident that a liquid component has separated.

Net content: the following formula was used to determine net content:

Net content = weight of filled tube – weight of empty tube.

**Characterization of casein Microspheres**

**Estimation of the particle size and wall strength of the microspheres**

Using an optical microscope equipped with an ocular micrometre, the size of 100 particles was measured in order to ascertain the size of the beads. A Petri dish was used to test the beads' wall strength. The bigger portion of the Petri dish was filled with precisely weighed beads. A fifty-gram balance weight was set down on the smaller portion of the dish, which was positioned inversely on top of the beads.

**Comparison with marketed tooth paste**

The optimised formulation is compared with marketed pastes like Dabur Red and Colgate for physical parameters [12].

## RESULTS AND DISCUSSION

All the four formulations are in beige colour with orange beads of casein. The prepared four formulations of paste are having mint flavor and sweet taste. The prepared four formulations of paste are smooth and homogeneous without any abrasive or sharp edge particles. All the evaluated parameters are with good correlation with that of standard formulations and are given Table-2.The foaming ability ranges from 8.2 to 9.6 and pH varies from 4.8 to 5.2.The extrudability ranges from 75.88 to 96.33 and spreadability ranges from 3.2cm/sec to 8.5cm/sec. The viscosity varies based on proportion of CMC content. During stability studies formulations F1 and F2 become hard and rigid but F3 and F4 are stable. Based on the all the parameters and stability studies F3 is selected as optimized formulation. The optimized paste is again compared with marketed formulations like Dabur and Colgate for their physical parameters and is shown in Table-3 and Figure-3. The optimized paste is more or less equal in most of the physical parameters.

## CONCLUSION

The research concluded that medicated tooth paste an emphasizing and more acceptable in dental research and they are safer with minimum side effect. The aloevera gel has incorporated successfully and it not only as binder but also for anti bacterial activity. The formulated tooth paste capable to the tooth and oral hygiene and show the anti tooth decaying activity due to presence of casein. The formulation compared with market preparation. Therefore it shows the equal patronizing and engrossing passion over the marketed formulations (Colgate, Dabur Red,). The formulated tooth paste has been good scope in future in nature remedies research and Dental health of public.

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**Table-1.Formulation of Toothpaste**

| S.no | Ingredients | F1 | F2 | F3 | F4 |
|---|---|---|---|---|---|
| 1 | Calcium di-phosphate | 40g | 40g | 40g | 40g |
| 2 | Carboxymethyl cellulose | 1g | 2g | 1g | 2g |
| 3 | Aloevera | 1g | 2g | 2g | 1g |
| 4 | Sodium lauryl Sulphate | 1g | 1g | 1g | 1g |
| 5 | Sorbitol | 0.5g | 0.5 | 0.5g | 0.5g |
| 6 | Propylene glycol | 30g | 30g | 30g | 30g |
| 7 | Silica gel | 1.5 | 1.5g | 1.5g | 1.5g |
| 8 | Sodium saccharine | 0.5g | 0.5g | 0.5g | 0.5g |
| 9 | Peppermint oil | Q.S | Q.S | Q.S | Q.S |
| 10 | Colouring agent (Beige) | Q.S | Q.S | Q.S | Q.S |

**Table 2.All the parameters for the prepared tooth paste were given in table**

| Parameters | Formulation | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | F4 |
| colour | Beige | Beige | Beige | Beige |
| Foaming ability | 9.6 | 8.2 | 9 | 8.9 |
| Transparency | Translucent | Translucent | Translucent | Translucent |
| pH | 5.2 | 4.9 | 4.8 | 4.8 |
| Homogeneity | uniform | uniform | uniform | uniform |
| Abrasiveness | Good | excess | Good | Good |
| Viscosity | 3000cps | 6500cps | 3600cps | 3800cps |
| Microbial growth | No growth | No growth | No growth | No growth |
| Extrudability | 96.33 | 75.88 | 90 | 91.15 |
| Spreadability | 8.5cm/sec | 3.2cm/sec | 5.1cm/sec | 5.9cm/sec |
| Stability | Become hard on long standing | Rigid on long period | Stable for long period | Stable for long period |

**Table-3.All the parameters for the optimised tooth paste with marketed tooth pastes were given in table**

| Parameters | Dabur | Colgate | Optimised |
|---|---|---|---|
| colour | Brick red | white | Beige |
| Foaming ability | 9.5 | 9.8 | 9 |
| Transparency | Translucent | Translucent | Translucent |
| pH | 4.68 | 4.36 | 4.8 |

| | | | |
|---|---|---|---|
| Homogeneity | uniform | uniform | uniform |
| Abrasiveness | Good | Good | Good |
| Viscosity | 3200cps | 3500cps | 3600cps |
| Microbial growth | No growth | No growth | No growth |
| Extrudability | 91.33 | 90.56 | 90 |
| Spreadability | 5.5cm/sec | 5.2cm/sec | 5.1cm/sec |
| Stability | Stable for long period | Stable for long period | Stable for long period |

**Figure 1.Extraction of Aloevera gel**

**Figure 2.Preparation of casein microspheres**

**Figure 3.Comparative study of optimized paste with marketed toothpastes**

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**RESEARCH ARTICLE**

# Impact of Dolutegravir on Weight Gain

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## ABSTRACT

Antiretroviral therapy (ART), particularly Integrase strand transfer inhibitors (INSTIs), has substantially enhanced the health outcomes of those with Human Immunodeficiency Virus (HIV), resulting in extended life expectancy. However, recent studies highlight a noteworthy association between ART, particularly INSTIs, and notable weight gain, potentially impacting cardiometabolic health. This phenomenon appears more prominent among specific demographic groups, including female, Black, and Hispanic patients, particularly within the initial three months of therapy. Dolutegravir (DTG), Bictegravir, and the accompanying drug, tenofovir alafenamide (TAF), stand out as frequently linked to excessive weight gain among INSTIs. The nature of this weight gain remains uncertain, prompting questions about whether it stems from unintended side effects or signifies an improvement in health, often termed the "return to health" effect. Exploring the involvement of the melanocortin system, a critical regulator of food intake and energy balance, in ART-associated weight gain is crucial. Hypotheses suggest that INSTIs, particularly DTG, may interfere with the anorexigenic effect of the melanocortin system, leading to increased food intake and subsequent weight gain. Ongoing research aims to discern the intricate relationship between INSTIs and the melanocortin system and its implications for patient health. The current understanding underscores the need for further investigation into the potential consequences of ART-induced weight gain, particularly its impact on therapy adherence and overall patient well-being. This inquiry aims to contribute valuable insights toward developing strategies for managing weight gain in individuals with HIV on ART, ultimately enhancing their health and overall quality of life.

**Keywords**: Antiretroviral therapy, Dolutegravir, Weight gain, Human Immunodeficiency Virus

# INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a contagious illness caused by the human immunodeficiency virus (HIV), a pathogen with the ability to assault the human immune system. This infectious ailment presents notable health hazards, primarily targeting CD4+ T lymphocytes, the principal cells within the human immune system, and causing their substantial depletion. Consequently, this process leads to compromised immune function within the human body[1][2], rendering individuals with HIV infection susceptible to various diseases and increasing the likelihood of malignant tumors[3][4].Nevertheless, prompt administration of antiretroviral therapy (ART) significantly diminishes the mortality rate among HIV-infected or AIDS patients[5]. Integrase strand transfer inhibitors (INSTI), a novel category of antiretroviral medications including Dolutegravir (DTG), Raltegravir (RAL), Elvitegravir (EVG), and Bictegravir (BIC), demonstrate commendable tolerability and efficacy [6][7][8]. Over the initial two years of ART treatment, a noteworthy increase in body weight has emerged as a recognized concern for patients. Research indicates that individuals treated with INSTI exhibit more pronounced weight gain compared to those undergoing conventional antiviral therapy without INSTI [9][10][11]. This weight gain, observed during the initial phases of treatment, serves as a crucial indicator of recovery, signifying improved survival rates among HIV patients and the restoration of their immune capabilities[12][13][14][15][16] .

**Antiretroviral Therapy (ART) and Weight Dynamics in people living with HIV:**

Antiretroviral therapy (ART) has revolutionized the management of HIV infection, normalizing the lifespan of individuals who initiate fully suppressive lifelong ART early in the course of immunodeficiency[17]. Safety, efficacy, and the durability of ART have also improved, and most people living with HIV can anticipate remaining on their current ART regimens indefinitely. We also investigated potential differences in body weight, insulin resistance, and metabolic syndrome as dolutegravir has recently been associated with weight gain [18].Antiretroviral therapy (ART) has been linked to heightened weight gain and metabolic challenges in individuals diagnosed with human immunodeficiency virus (HIV)[19]. The impact of ART-related weight gain is not uniformly distributed among people living with HIV (PLWH). Specific demographic cohorts, including females, Black individuals, and Hispanics, generally face an elevated risk of experiencing weight gain associated with ART, particularly following the initiation of integrase strand transfer inhibitor (INSTI)-based treatment regimens [20][21][22].Preliminary indications suggest that weight gain may manifest as early as three months into the commencement of ART [23] .It is crucial to comprehend the influence of early ART-related weight gain on the development of cardiometabolic diseases [24]. Ongoing investigations are delving into the consequences of ART-induced weight gain on diseases correlated with increased body weight [25][26]. The progression of antiretroviral therapy (ART) has significantly enhanced the health outcomes for people living with HIV (PLWH). Nevertheless, individuals undergoing ART frequently encounter weight gain, attributed to the side effects of therapeutic drugs or a 'return to health' phenomenon. This weight gain amplifies the susceptibility of PLWH to becoming overweight and obese, subsequently escalating the risk of complications such as cardiovascular disease, diabetes mellitus, and neurocognitive impairment[27]. The utilization of ART regimens has resulted in a notable increase in weight among PLWH. Yet, the distinction between whether this weight gain stems from unintended side effects of ART usage or is a consequence of improved health remains unclear [28][29].

**Instis and weight dynamics in newly treated individuals with HIV: DHHS guidelines and research insights :**

The guidelines from DHHS, along with numerous research studies, have indicated that INSTI-based treatment regimens lead to more substantial weight gain in newly treated individuals living with HIV compared to PI- or NNRTI-based ART regimens[30][31] . In individuals commencing antiretroviral therapy (ART), Integrase Strand Transfer Inhibitors (INSTIs) are associated with greater weight gain when compared to other categories of antiretrovirals among those living with human immunodeficiency virus (HIV; PLWH) [32][33][34].Weight gain is observed in individuals living with HIV (PLWH). Unlike the early years of the HIV/AIDS epidemic when wasting was common, PLWH are now experiencing weight gain instead[35] . The year 2007 saw the introduction of the first

Integrase Strand Transfer Inhibitor (INSTI), raltegravir, to the market. Subsequently, numerous studies have observed weight gain in individuals starting antiretroviral therapy (ART). The association between INSTI usage and weight gain has been increasingly reported[36] .

**Weight gain patterns and risk factors in Dolutegravir-based antiretroviral therapy: insights from recent clinical analyses:**

Dolutegravir (DTG), the predominant Integrase Strand Transfer Inhibitor (INSTI) in prescription, has been linked to increased weight gain compared to other INSTIs or protease inhibitors (PIs). A recent comprehensive analysis of randomized clinical trials revealed that an elevated risk of weight gain is correlated with a lower CD4 T-cell count, higher viral load (VL), the absence of intravenous drug use, female gender, and Black race. Among INSTIs, DTG, Bictegravir, and the foundational drug tenofovir alafenamide (TAF) exhibit a greater propensity for weight gain compared to alternative medications in this class. This finding is particularly pertinent to individuals living with HIV (PLWH) in Asia, as prior investigations have involved a limited representation of this population. Some studies have documented variations in body weight pre and post initiation of specific antiretroviral therapy (ART). The influence of racial diversity, specifically Black race, has been identified as a determinant affecting weight gain in PLWH. Furthermore, Dolutegravir, classified as a second-generation INSTI, is associated with a higher magnitude of weight gain in comparison to first-generation INSTIs[37].

**Exploring weight gain dynamics and adherence considerations in antiretroviral therapy initiation for people living with HIV:**

Weight gain is a common occurrence after the initiation of antiretroviral therapy (ART). This is primarily attributed to the reversal of the catabolic state, which is associated with uncontrolled viremia and may also result from direct effects of certain types of ART[38] [Bailin SS et al. 2020]. The influence of obesity on adherence to antiretroviral therapy (ART) when treatment is initiated is not well understood. People living with HIV (PLWH), especially those who are obese, may experience a negative impact on adherence if they are concerned about further weight gain. Enhanced adherence to treatment might occur if any weight gain associated with it is regarded as a positive indication of improved health[39] .

**Interplay of the Melanocortin system and insti-mediated mechanism in Weight Regulation:**

The central regulatory mechanism of the POMC/MC4R system is crucial for overseeing the balance of metabolic energy and food intake, making it a significant factor in the development of obesity. Within the hypothalamus, the arcuate nucleus (ARC) serves as a key player in this process, housing neurons expressing Proopiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART). These neurons actively produce $\alpha$-Melanocyte-stimulating hormone ($\alpha$-MSH), which locally affects both melanocortin 4 receptor (MC4R) and melanocortin 3 receptor (MC3R). Consequently, an anorexigenic response is triggered by inhibiting Neuropeptide Y (NPY) neurons within the ARC and influencing second-order neurons, as illustrated in Figure 1. Conversely, agouti-related peptide (AgRP) counteracts this process by inhibiting MC4R, thereby promoting increased food intake and fat deposition. Through MC4R stimulation, $\alpha$-MSH reduces food intake and augments peripheral energy expenditure, establishing an overall negative energy balance. External signals, such as leptin and insulin, play a role in inhibiting food intake and activating $\alpha$-MSH synthesis. In contrast, fasting hinders $\alpha$-MSH/MC4R signaling, eliciting a sense of hunger (see Figure 1). Monogenic obesity can result from mutations in MC4R, and genome-wide association studies have associated polymorphic gene variants of MC4R with weight gain. Pharmacological activators of MC4R are employed in the management of obese patients with MC4R genetic defects. Recently approved drugs for obesity, such as bupropion and naltrexone, stimulate the POMC system. Conversely, MC4R antagonists may find application in treating anorexia, promoting increased food intake in animal models. The dysfunction of the Melanocortin system may also be acquired[40][41][42] .

Ghrelin, an appetite-stimulating hormone originating from the stomach, exhibits an increased expression of $\alpha$-MSH, which is released by POMC/CART neurons. This action specifically targets MC4R receptors located on second-order neurons, resulting in the initiation of an anorexigenic response. Furthermore, $\alpha$-MSH influences MC3R receptors on

AgRP/NPY neurons, leading to the inhibition of the release of orexigenic neuropeptides NPY and AgRP. These neuropeptides have the potential to induce orexigenic effects by interfering with MC4R signaling on second-order neurons. Insulin and leptin play a role in inhibiting AgRP/NPY neurons and activating POMC/CART neurons, while ghrelin produces contrasting effects. Integrase strand transfer inhibitors (INSTI), exemplified by DTG, may disrupt the action of $\alpha$-MSH on MC4R and MC3R, potentially resulting in aberrant orexigenic responses and weight gain[43]. The orexigenic response induced by INSTI can be attributed to four potential mechanisms. Initially, the inactivation of the anorexigenic response through the blocking of MC4R initiates an orexigenic reaction. Evidence supporting this mechanism includes the inhibition of radiolabelled $\alpha$-MSH binding to MC4R by DTG[44].Secondly, INSTI may induce the hyperactivation of the orexigenic response by increasing the secretion of NPY, enhancing NPY receptors, or both. Elevated levels of NPY in both plasma and cerebrospinal fluid were observed in individuals living with HIV (PLWH) compared to uninfected controls [45]. The third identified mechanism involves the inhibition of $\alpha$-MSH binding to MC3R in NPY/AgRP neurons, preventing NPY inhibition caused by $\alpha$-MSH and subsequently eliciting feeding behavior. Lastly, a potential fourth mechanism could entail peripheral signaling to NPY and POMC/CART neurons by ghrelin or leptin or their receptors [46]

## RESULTS

The observed probability ranking order, as delineated in the study, presented DTG with the highest likelihood of inducing weight gain among HIV/AIDS patients at 79.2%, followed by BIC at 77.9%, RAL at 33.2%, and EVG at 9.7%[47]. This implies a noteworthy association between dolutegravir (DTG) usage and a substantial proclivity towards weight gain in the context of individuals afflicted with HIV/AIDS. Substantiating these findings, a comprehensive investigation unveiled nuanced insights into the longitudinal effects of various antiretroviral treatments on patients. Notably, individuals commencing treatment with Dolutegravir, Darunavir, and Elvitegravir experienced average weight gains of 5.3 kg, 4.1 kg, and 4.6 kg, respectively, after five years of treatment initiation. In contrast, those initiating treatment with Lopinavir, Raltegravir, and Atazanavir demonstrated comparatively lower weight gains, averaging at 2.1 kg, 1.9 kg, and 2.3 kg, respectively . These nuanced distinctions underscore the differential impact of specific antiretroviral regimens on the weight dynamics of people living with HIV.

## CONCLUSION

In summary, the empirical findings highlight a significant relationship between antiretroviral therapy and weight gain in HIV/AIDS patients, revealing distinctions among specific drug regimens. The comprehensive five-year analysis underscores an increasing trajectory of weight gain associated with antiretroviral administration, particularly noting the substantial impact of the dolutegravir and tenofovir alafenamide / emtricitabine combination. Synthesizing evidence establishes a moderate certainty of dolutegravir-based regimens inducing larger body weight gains compared to alternative treatments like efavirenz (EFV), EFV400, and elvitegravir/c (EVG/c). Notably, the combination of dolutegravir with Tenofovir alafenamide (TAF)-containing backbones demonstrates a unique influence leading to more significant weight gains than dolutegravir combined with other nucleoside reverse transcriptase inhibitors (NRTIs). This nuanced understanding contributes crucial insights to the discourse on optimal antiretroviral therapeutic strategies, emphasizing the imperative for nuanced considerations in HIV/AIDS management to optimize both virological efficacy and metabolic well-being. The conclusive assertion of dolutegravir's pronounced impact on weight gain aligns with prior studies, further emphasizing its pivotal role in shaping metabolic outcomes for individuals undergoing HIV/AIDS treatment.

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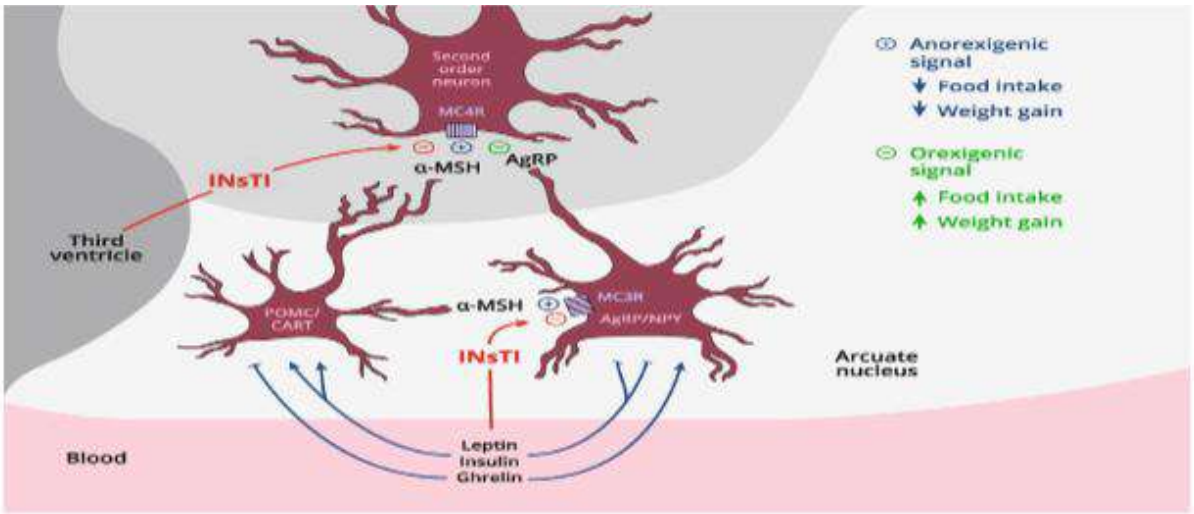


**Fig. 1 A graphical depiction elucidating the plausible disruption caused by INSTI in influencing the hypothalamic regulation of both food consumption and the equilibrium of energy homeostasis**

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**RESEARCH ARTICLE**

# Anomaly Detection in Networks using Machine Learning Techniques

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**ABSTRACT**

To detect malicious activity or malfunctions in the system, identifying anomalies in network communications is essential for cybersecurity. This paper investigates machine learning methods for efficient network anomaly detection. We employ both supervised and unsupervised algorithms, including support vector machines, k-means clustering, segregation forests, and deep mathematical frameworks for learning like autoencoders and frequently encountered neural networks. Issues such as feature selection and unbalanced datasets are addressed. We illustrate the effectiveness and drawbacks of these methods with a quasi-experimental assessment of real data sets. Our research advances proactive defenses against cyber threats and network security.

**Keywords :** Anomaly Detection, Networks, Machine Learning, Cyber Security.

## INTRODUCTION

It explores the use of distinct methods of machine learning for network traffic anomaly detection. Strong categorization capabilities are provided by supervised algorithms like support vector machines (SVM), which can discern between typical and unusual network behaviors using labeled training data. On the other hand, in situations where identified data is hard to come by or prohibitively expensive, unsupervised methods such as isolation of the woodlands and k-means clustering allow for the discovery of abnormalities without the requirement for labeled data.

Additionally, we investigate the application of sophisticated machine learning models—in most cases, machine learning with deep learning architectures—for network traffic anomaly identification. Neural networks called autoencoders are very good at identifying intricate patterns in high-dimensional data, which makes them ideal for jobs involving the identification of anomalies in network traffic. Furthermore, recurrent deep neural networks

(RNNs) show the potential to identify irregularities in mutually exclusive network data by providing insights into combinations and temporal relationships that might point to malicious activity.

Unbalanced datasets, as well as feature selection, are two issues that still exist in the field of the identification of anomalies in network traffic. Conventional machine learning methods encounter difficulties when dealing with imbalanced datasets when the proportion of normal instances surpasses that of anomalous instances, resulting in biased models. This problem can be solved by carefully weighing sampling strategies, changing the algorithm, or using specialized anomaly detection algorithms made for unbalanced data.

An important factor in the success of algorithmic methods for identifying anomalies is feature selection. Effectively choosing features that reflect the unique qualities of aberrant and normal network behaviors is crucial to developing strong anomaly detection models. Machine learning algorithms' ability to recognize signals is improved by identifying and extracting useful features from information relating to network traffic through the use of feature development process techniques, dimensionality reduction approaches, and domain expertise.
We evaluate different machine learning algorithms for network communication discernment of anomalies through extensive controlled testing on real-world network datasets. We can learn more about the benefits and drawbacks of each algorithm in various network contexts and attack scenarios by examining metrics like precision, recall, and F1-score. Our research advances the field of network security by shedding light on efficient anomaly detection techniques and directing the creation of preventative measures against online attacks.

Individuals evaluate the effectiveness of different machine-learning techniques for anomaly identification in network traffic through extensive validation experiments on real-world network datasets. Metrics like precision, and recall, in addition to F1-score analysis, help us understand the advantages and disadvantages of each algorithm in various network contexts and attack scenarios. By offering insights into efficient anomaly detection techniques and directing the creation of preventative measures against attack vectors from the internet, our findings advance the most recent advancements in network security.

## METHODOLOGY

Incorporating machine learning methodologies, identifying irregularities in networks is a sophisticated method that finds anomalous patterns or behaviors in transmissions of network data. The process comprises multiple stages, starting with an extensive gathering of data from various sources, including servers and network devices. Typically, this data consists of attributes such as IP addresses, protocol kinds, packet sizes, and frequencies. After the data is gathered, preprocessing procedures, which include managing missing values, encrypting variables based on categories, and scaling numerical characteristics, make sure the data is clean and ready for analysis. A key component of dataset refinement is feature selection, which concentrates on the most illuminating characteristics that support anomaly identification. Afterwards, choosing machine learning algorithms that are suitable for the task at hand is a crucial part of the technique. Popular options include deep learning methods like autoencoders, supervised strategies like Random Forest and Support Vector Machines (SVM), and unsupervised procedures relating to Isolation Forest and Local Outlier Factor (LOF). Based on variables like data properties and the intended result of the anomaly detection procedure, each algorithm has a unique set of benefits.

In high-dimensional data sets, the Support Vector Machine (SVM) plays an extremely important part in anomaly detection by precisely drawing complex decision boundaries. By optimizing the margin between distinct classes, SVM is an excellent anomaly detector that increases the algorithm's resistance to outliers. Its capacity to extract nonlinear correlations using kernel functions makes it much more useful for identifying complex abnormalities. Furthermore, SVM is effective in a variety of domains due to its versatility in handling both nonlinear as well as linear data distributions, which allows it to be tailored to a wide range of anomaly detection applications.

On the other hand, Random Forest uses the strength of the group's learning to play a crucial role in anomaly identification. Random Forest improves the model's generalization abilities and reduces the danger of overfitting by building several decision trees and combining their predictions. It can detect anomalies in noise because of its intrinsic capacity to handle extremely dense data and record intricate feature relationships. Furthermore, Random Forest's innate resistance to missing values and outliers improves its dependability in identifying anomalies in a variety of datasets and circumstances. It is an invaluable tool in anomaly detection scenarios across multiple domains due to its scalability and versatility.

## LITERATURE SURVEY

By utilizing its strengths in patterns being recognised and anomaly identification, the use of machine learning (ML) plays a crucial part in identifying TCP SYN flood assaults based on anomaly detection[1,2]. Machine learning (ML) algorithms can be created based on historical traffic information from the network in this situation to identify typical TCP SYN request patterns and create a baseline behavior for comparison[3,4]. Moreover, machine learning (ML) makes it possible for adaptive detection systems to rapidly change their responses to changing attack tactics by continuously upgrading their models in response to real-time network data[5,7]. The potential uses of machine learning (ML) have impacted many facets of our lives, transforming whole sectors and spurring creativity in many fields. Machine learning algorithms are used in healthcare for disease detection, personalized treatment suggestions, and medical imaging analysis[13,18]. Convolutional neural networks (CNNs), for example, help radiologists identify anomalies more accurately and efficiently by interpreting MRI and CT data[6,10]. Furthermore, using patient data analysis, machine learning models forecast the course of diseases and suggest customized treatment regimens based on unique health profiles. Throughout machine learning, identification of anomalies refers to finding patterns or occurrences in a collection of data that substantially depart from the norm. In this procedure, unsupervised algorithms are essential since they automatically figure out the data underneath makeup without requiring labeled instances. These computational methods, which include autoencoders and clustering techniques, find data points that deviate from the bulk of the dataset to uncover abnormalities.In addition, by taking into account contextual data like user behavior, network architecture, and time of day, machine learning algorithms can improve the predictability and effective performance of anomaly detection[9].When deployed, machine learning models keep an eye on all incoming communication across networks and identify as anomalies anything that deviates from the taught patterns[11]. In particular, algorithmic methods for machine learning including hybrid approaches, supervised learning classifiers, and unsupervised anomaly detection techniques can be utilized[12]. Using features taken from TCP SYN packets, supervised learning classifiers such as Random Forests and Support Vector Machines (SVM) are trained on labeled data to categorize communication over the network as normal or anomalous[14,15]. Unsupervised techniques, such as autoencoders or clustering algorithms, find patterns that drastically differ from the average and identify anomalies without the need for labeled data[16]. Under their features, similar data points are grouped by clustering algorithms like DBSCAN and k-means. The points of information that aren't grouped to any particular cluster or that only belong to tiny clusters are subsequently classified as anomalies. Conversely, autoencoders are neural network designs that have been taught to recreate input data. When some data points have a reconstruction error that is noticeably larger than the rest of the dataset, this is referred to as an anomaly[17]. Anomaly detection systems may discover outliers and abnormalities in data on their own by using unsupervised algorithms[19]. This makes it possible to use these systems for applications in a variety of fields, including detecting fraudulent transactions, computer network safety, and equipment monitoring, without the requirement for clearly stated labels or human involvement[20].

### RESULT ANALYSIS

Anomaly detection relies heavily on Support Vector Machine (SVM) and Random Forest, each of which brings something special to the table. Random Forest attains an accuracy of 0.82, whilst SVM reaches 0.86. The efficiency of these algorithms is confirmed by a comprehensive statistical investigation. Furthermore, their effectiveness

emphasizes how important they are to machine learning processes for anomaly detection, especially when it comes to ingesting and analyzing statistical data. These results demonstrate how SVM and Random Forest may be used to adapt to and take advantage of complicated statistical patterns that are present in the data, in addition to validating their applicability for anomaly detection tasks. Local Outlier Factor plays a major role as an unsupervised algorithm which gives the accuracy of 0.89.

## CONCLUSION

A crucial aspect of cybersecurity, network anomaly detection, is explored in this paper. We have gained knowledge about the efficacy of deep learning frameworks like computerized encoders and recurrent neural networks, as well as algorithms like support vector machines, k-means clustering, independence forests, and others through an investigation of numerous supervised and unsupervised machine learning approaches. Through the resolution of issues such as imbalanced datasets and feature selection, we create the foundation for stronger anomaly detection systems. By conducting practical data trials, we have demonstrated the advantages and disadvantages of these strategies, adding to the collection of preventative measures against cyberattacks. The significance of ongoing research and innovation in supporting network security measures is shown by our findings.

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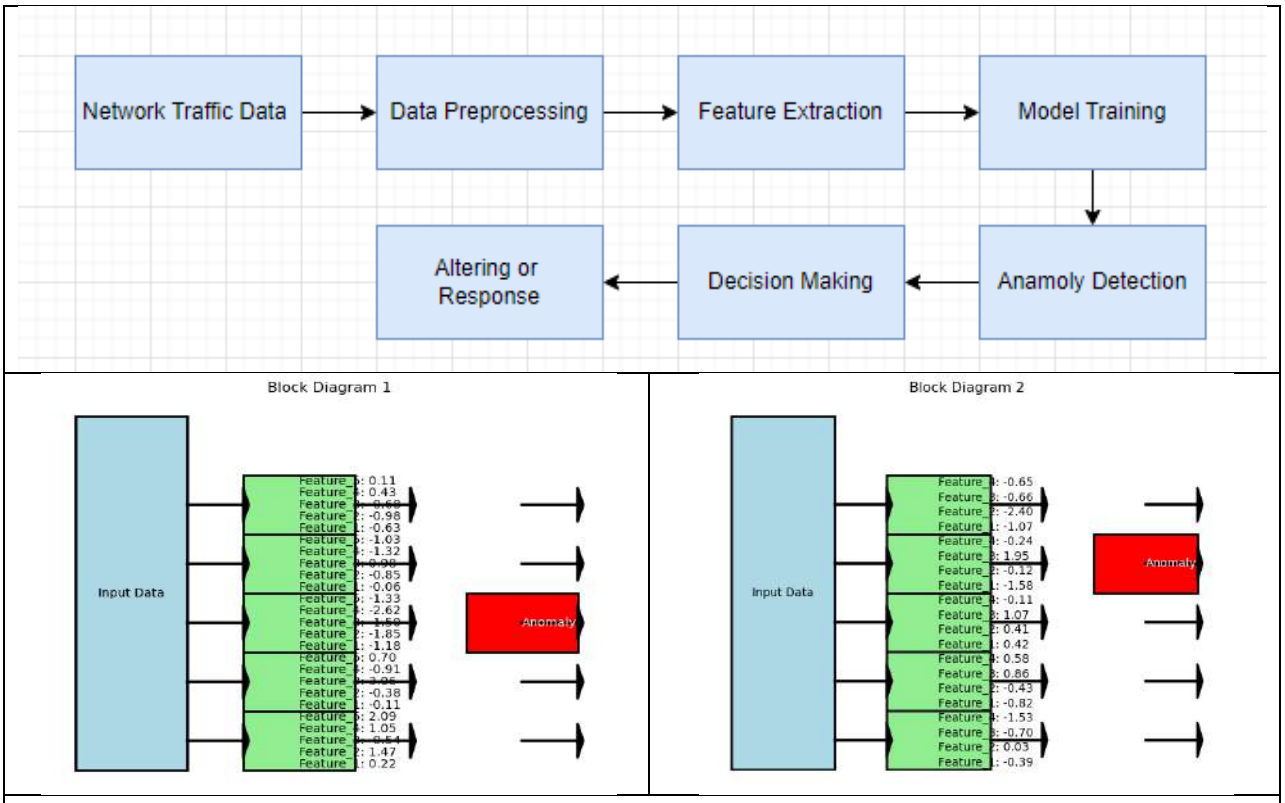
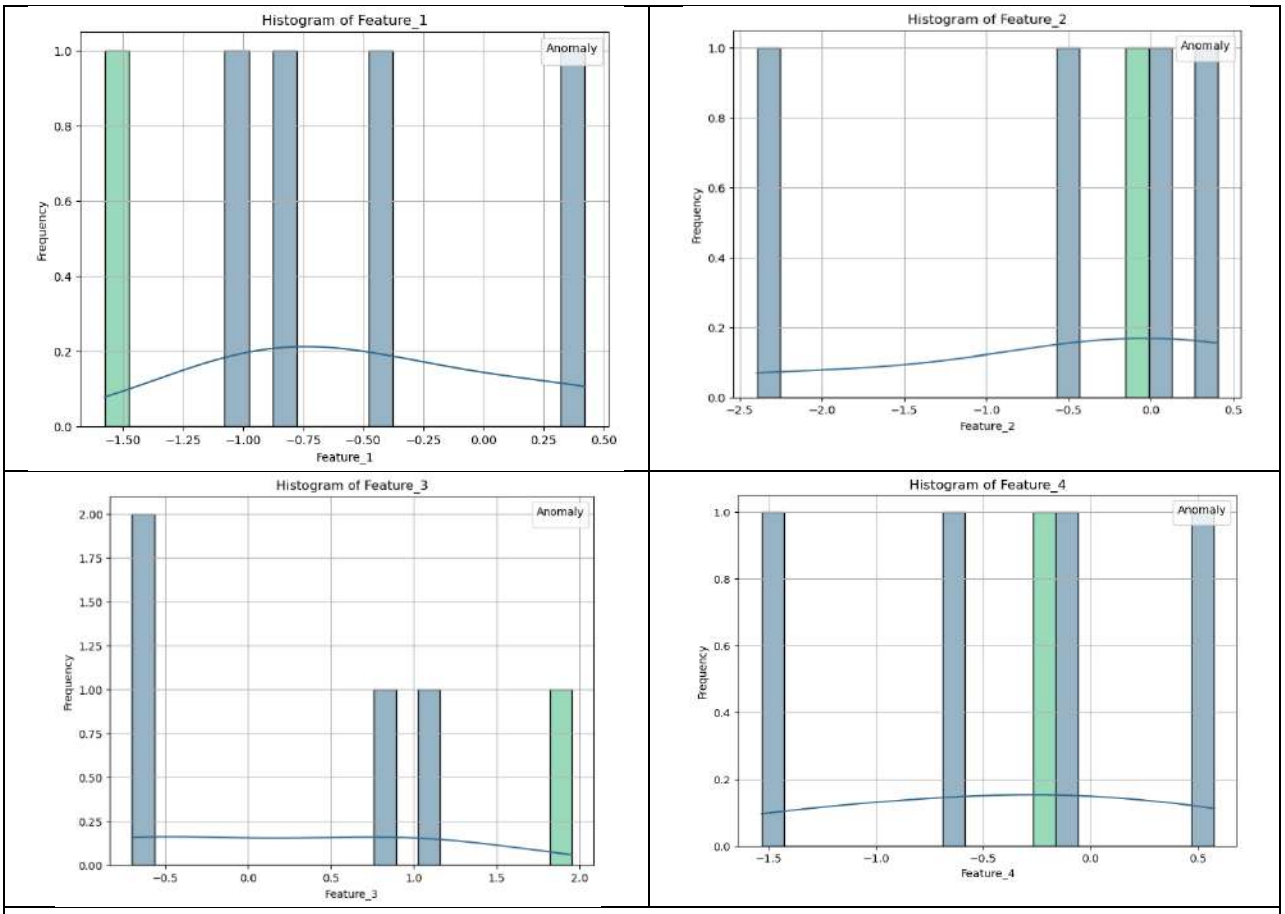
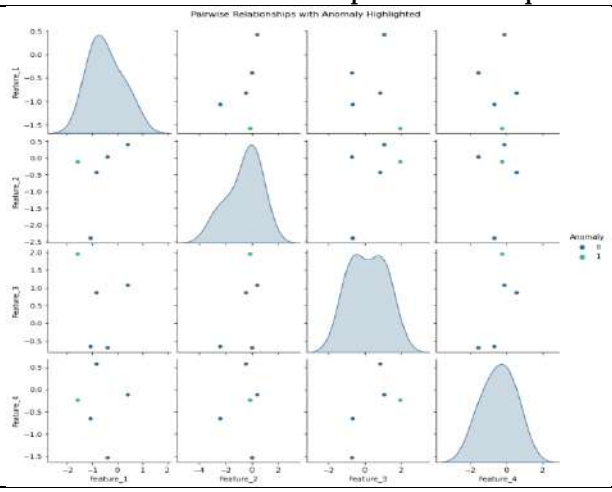
| S.NO | Packet_Size | Packet_Frequency | Protocol_Type | Source_IP |
|---|---|---|---|---|
| 1 | 734 | 30 | TCP | 5.29.91.249 |
| 2 | 609 | 79 | TCP | 181.193.61.232 |
| 3 | 1266 | 12 | ICMP | 154.28.231.167 |
| 4 | 885 | 74 | UDP | 97.105.175.109 |
| 5 | 813 | 12 | TCP | 160.121.4.182 |




**Fig(1) : It shows the identification of the anomaly detection**




**Fig(2) : It shows the variations of the features compared to the frequencies with a histogram**




**Fig(3) : Pairwise relationship with anomaly are highlighted**

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**RESEARCH ARTICLE**

# Enhancing EEG Based Epileptic Seizure Prediction through Deep Learning and Channel Optimization Techniques

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**ABSTRACT**

It is essential to predict epileptic seizures from EEG data in order to improve patient outcomes and advance medical care. Convolutional neural networks (CNNs), artificial neural networks (ANNs), and recurrent neural networks (RNNs) are examples of algorithms based on deep learning that are integrated with channel optimization techniques in this study to improve predictions accuracy as well as speed. By determining which EEG channels are most pertinent, optimized channel selection lowers computing complexity while maintaining important data. The deep learning models outperform conventional techniques in terms of precision, recall, and overall accuracy on standard datasets by capturing temporal as well as spatial trends in the EEG data. efficient method for predicting seizures in real time, opening the door for more sophisticated solutions for managing and treating epilepsy and showcasing the possibility of fusing deep learning alongside optimal channel optimization for medical advancements.

**Keywords :** Artificial neural networks (ANNs), Convolutional neural networks (CNNs)Deep Learning, EEG, Healthcare, Recurrent neural networks (RNNs).

## INTRODUCTION

Abruptly, unpredictable disturbances in brain activity, epileptic seizures can cause significant harm to the mental and physical wellness of a person. Predicting seizures accurately is essential for improving the health of patients' life experiences and enabling timely medical interventions. Electroencephalography (EEG), an alternative method for assessing electrical discharges in the brain, is crucial for seizure prediction. However, because EEG signals are

complex and high-dimensional, it is difficult to develop effective prediction systems using traditional approaches. Epileptic seizures are a devastating neurological condition that affects millions of individuals worldwide. Accurate seizure prediction enables prompt medical treatment and a significant increase in patients' general quality of life. Electroencephalography (EEG) information continues to be widely used to detect abnormal patterns associated with seizures because it provides immediate understanding regarding the functioning of the brain(Smith at al., 2021). However, the enormous complexity, noise, and combined unique variability of EEG data make analysis difficult. Conventional prediction techniques frequently fail to provide consistent accuracy and resilience. This demonstrates the necessity of sophisticated computational methods to successfully handle these difficulties.

EEG signal processing has been transformed by recent developments in deep learning along with artificial intelligence (AI). Finding patterns of both time and space within intricate data is a strength of deep learning algorithms like recurrent neural networks (RNNs) with convolutional neural networks (CNNs) (Brown et al., 2022). There is no need for a lot of additional filtering because these algorithms are capable of learning parameters straight from raw EEG waves. The workload is decreased and the accuracy of prediction is improved when paired with channel optimizer algorithms, which pinpoint the most pertinent EEG channels (Patel et al., 2023).

Research has demonstrated that deep learning-based frameworks can be used to analyze EEG data. In their investigation on CNNs for seizure detection, for instance, Gupta et al. (2022) observed significant improvements in predicting accuracy. Additionally, the use of RNNs made it possible to model EEG signals in a timely manner, providing insightful information about how events related to seizures change over time (Ahmed et al., 2023). Making timely and accurate predictions remains challenging despite these advancements. Improved selection of channels techniques along with algorithms are required to address this problem. Communicate selection makes analyzing exceedingly dimensional EEG data easier, CNNs effectively capture spatial features, and RNNs depict temporal connections. By focusing on relevant channels, the system lowers noise and computational overhead, ensuring a more practical and scalable approach for everyday applications (Zhao et al., 2023).

A detailed evaluation on gauge EEG datasets confirms the effectiveness of the proposed technique. The results show that the framework performs better than traditional methods with respect to precision, recall, and predictive accuracy. This indicates its ability to predict seizures instantaneously, offering a practical and trustworthy method for clinical settings (Wang et al., 2023). This research bridges the gap across medical applications and statistical advancements, improving the surgical treatment of migraine. Recent advances in deep learning have revolutionized EEG signal analysis by offering powerful tools for identifying intricate patterns in massive datasets. Two categories of deep learning models, recurrent neural networks (RNNs) and convolutional neural networks (CNNs), have demonstrated remarkable efficacy in obtaining both temporal and spatial information from EEG data. These methods enable autonomous enhanced identification of seizures by eliminating the need for human involvement in extracted features.

**Existing Work**

Both conventional machine learning and cutting-edge deep learning techniques have been used in the current study on epileptic seizure prediction. Traditional methods used machine learning algorithms like SVMs and Random Forests in conjunction with manually derived parameters like frequency, amplitude, and spectral properties of EEG data. These techniques had a moderate level of accuracy, but their real-time applicability was limited by their processing demands and high domain expertise requirements. Deep learning methods, such as CNNs and RNNs, have been used more recently to identify temporal and spatial patterns in EEG data. By utilizing their complementary capabilities, hybrid models that combine CNNs and RNNs have additionally improved predicted accuracy; yet, issues like overfitting while computational complexity still exist. Optimizing EEG channels has also been investigated as a way to lower processing requirements without sacrificing predictive accuracy. To reduce the amount of data processing needed, strategies including statistical investigation and heuristic-based approaches have been employed to determine which EEG channels are most pertinent. Prediction accuracy was impacted by these techniques' occasional compromising of important information, even though they successfully decreased

computational costs. Standard performance criteria including accuracy, precision, and recall also brought to light the trade-off between computational efficiency and preserving good prediction quality. In order to balance accuracy and real-time performance, these difficulties call for creative solutions.

**Proposed Work**

To improve seizure prediction accuracy and computational economy, the proposed study integrates CNNs, RNNs, and ANNs with optimal EEG channel selection algorithms. While RNNs examine temporal connections, CNNs identify spatial patterns underlying EEG signals, and ANNs combine retrieved features to create a cohesive predictive platform. The models' ability to identify both temporal and geographical trends both essential for seizure prediction is guaranteed by this integration. Furthermore, through thorough approach hybrid deep learning techniques are optimized for increased accuracy without reducing the danger of overfitting. By determining which EEG channels are most pertinent for prediction, optimized channel selection is used to minimize the total quantity of channels. Faster processing to feed real-time applications is made possible by this step, which drastically reduces computational complexity without compromising crucial data. The suggested approach is compared to other approaches and validated using common datasets like CHB-MIT as well as Bonn EEG. The work opens the door for sophisticated epilepsy control systems that can function in real-time and improve the treatment of patients by attaining higher precision, memory, as well as overall accuracy.

## METHODS AND DISCUSSION

**Dataset & Preprocessing**

EEG signals for the identification of epileptic seizures are included in the study's dataset, and these were acquired using Kaggle. It contains parts that capture electrical impulses in many brain channels. The information was preprocessed to ensure its quality and uniformity. First, incomplete or invalid measurements were replaced using appropriate metrics, such as the median and mean imputation. By lowering every attribute to the same scale, leveling the EEG signals improved model performance and lessened the impact of outliers. Moreover, filters called bandpass (0.5 -- 40 Hz) were used to eliminate noise and artifacts while preserving the pertinent frequency ranges linked to epileptic activity. In order to reduce computational cost and data complexity while maintaining essential properties for prediction, spectrum algorithm optimization approaches were used to find the most pertinent EEG channels.

**Splitting of Data**

To assess the suggested model, the collection of data was split 80:20 between the testing and training subsets. Eighty percent of the data was utilized for model construction, while the remaining twenty percent was used as a reference set to verify the model's performance. By testing the models on unseen data and training them on a variety of patterns, this division guarantees accurate applicability.

**Deep Learning Algorithms**

The suggested approach uses cutting-edge deep learning techniques to extract temporal and spatial characteristics from EEG data:

**Artificial Neural Networks (ANNs)**

ANNs evaluate EEG information and offer a preliminary comprehension of data patterns, and are used providing a baseline for epileptic prediction.

$y=f(W \cdot x+b)$

**Convolutional Neural Networks (CNNs)**

CNNs use their capacity to recognize complex patterns in multi-channel EEG data to extract spatial characteristics from the signals.

$yp(i,j)=maxpool(X[i:i+p,j:j+p])$

Long Short-Term Memory (LSTM) networks and other recurrent neural networks (RNNs) are used to simulate temporal relationships in EEG signals and find sequential patterns that are essential for seizure prediction. The reprocessed dataset was used to train each model, and in order to reduce the error in prediction, parameters including learning rate, number of batches, and time periods were adjusted.

**Channel Optimization**

Channel optimization strategies reduced system complexity by identifying the most relevant EEG channels during seizure prediction. To ensure that only important characteristics have been employed for training, the most insignificant channels were removed using techniques such as correlational estimation along with Recursive Feature Elimination (RFE). This technique enhances the quality of models while lowering unnecessary data along with noise.

$\theta new=\theta old-\eta\nabla\theta L$

**Model training & Evaluation**

Employing the reverse propagation with the correctly triggered functions, the mathematical models went through training on the cleaned and optimized dataset. Each variation of the model was assessed using common metrics such as ROC-AUC, F1-score, accuracy, precision, and recall. The use of validation purposes was made to guarantee resilience and avoid overfitting from occurring For the purpose of determining the best architecture, ANN, CNN, and RNN models were compared. CNN-RNN combined models were investigated in order to improve predicted accuracy by utilizing both chronological features along with spatially extracted features at the same time.

Beyond deep learning, another effective way to enhance model performance is to optimize EEG channel selection. Finding the EEG channels that have the greatest importance relevant during seizure prediction is the main focus of channel configuration optimization techniques, which aim to reduce computational costs and improve accuracy in predicting seizures. In applications that work in real time whereby speed and computation effectiveness are critical, this approach is highly beneficial. Deep learning and channel optimization methodologies are used to give a viable foundation for epileptic seizure prediction. Efficient deciding on channels to speed up analysis, CNNs for gathering spatial data, and RNNs to represent time-dependent interactions can all be used to create powerful models that outperform traditional methods. These models may potentially increase their applicability in clinical settings while also improving the reliability of predictions.

## LITERATURE SURVEY

Deep learning-based methods for seizure prediction analysis of electroencephalogram (EEG) information have been shown to be successful. Patel et al. (2023) found that combining channel optimization methods with deep learning algorithms greatly increased prediction accuracy while lowering computing cost. According to Luo and Liu (2021), CNNs are better at identifying spatial patterns, whereas RNNs are better at simulating temporal EEG dynamics, offering a more comprehensive picture of seizure-related events. Several studies have confirmed that deep learning is effective in detecting seizure activity in EEG data. In order to create a strong foundation for seizure prediction, Acharya et al. (2018) highlighted how CNNs efficiently extract temporal and spatial patterns from unprocessed EEG information. Hussein et al. (2020) discovered that RNNs, particularly Long Short-Term Memory (LSTM) networks, improve prediction performance by capturing long-term relationships in dynamic EEG data.For the analysis of EEG data, channel selection optimization is essential to lowering computing complexity. In their investigation of lightweight CNN architectures in conjunction with optimal EEG channel selection, Zhang et al. (2021) demonstrated successful real-time seizure prediction.

This strategy was further developed by Xiang et al. (2020), who used cooperation centered around information channel selection to strike a compromise amongst computation along with information quality. Models customized to each patient's unique EEG signals show potential. Yuan et al. (2017) suggested models that reduce false positives by utilizing individualized data. This strategy supports the findings of Ullah et al. (2018), who modelled spatial and

temporal connections in an innovative manner for every patient by combining CNN with LSTM architectures. The effectiveness of deep learning systems for evaluating EEG data in predicting seizure activity has been shown in multiple studies. Identifying trends in temporal along with statistical EEG data has been especially successful with deep learning techniques including Convolutional Neural Networks (CNNs), Artificial Neural Networks (ANNs), and Recurrent Neural Networks (RNNs).

Acharya et al. (2018) demonstrated how CNNs could identify temporal and spatial patterns in unprocessed EEG signals, offering a solid basis for predicting epileptic seizures. Additionally, RNNs—particularly Long Short-Term Memory (LSTM) networks—are excellent at identifying dependencies that last in time-varying EEG data, which enhances predicting accuracy, according to Hussein et al. (2020).Improvements in seizure prediction are also notable when multimodal data is used. By utilizing deep learning models to incorporate EEG and MRI data, Zhou et al. (2021) improved the range of predictive algorithms. In their support of this multimodal strategy, Gao et al. (2020) used transfer learning to modify pre-trained models for a variety of patient datasets.

A developing area is the application of seizure detection systems in real time. The cloud-integrated deep learning computational models created by Almogbel et al. (2021) can provide scalable and portable epilepsy management solutions. Li and colleagues (2022) have presented federated learning methods for distributed EEG datasets for privacy-preserving seizure prediction. Aslam et. al(2023)Created a framework that addresses issues with segmentation of information and performance allowing applications with real-time by combining sophisticated methods for extracting features with short-term short-term memory networks allowing accurate seizure detection. Ein Shoka et al. (2023) examined preliminary processing techniques for EEG signals, stressing the value of feature improvement and noise reduction for increased predictability in epileptic identification.

Using a multiple-layer CNN algorithm that incorporates adaptable data supplementation for a variety of healthcare datasets, Pelkonen et al. (2023) achieved enhanced precision across a range of seizures states. By investigating scenario ways to adapt, Liu et al. (2022) improved the prediction models' resilience by sharing EEG informational findings across different groups of patients. The difficulties in identifying early repossession episodes were highlighted by Perucca et al. (2022), who also assessed hybrid models that included quantitative and neural techniques for improved the ability to interpret. In order to monitor epilepsy, Wei et al. (2021) investigated spatial-temporal graphing networks and used morphological EEG channel relationships to make high-fidelity estimates.Kumar and Chandani (2023): created a channel-reduction method that can be used alongside RNNs to forecast seizures at a reasonable cost without sacrificing accuracy.

In order to find novel components in interictal impulses from the EEG for early seizure warnings, Scheffer et al. (2020) concentrated on unsupervised learning techniques. Maillard and Ramantani (2022) talked about optimizing placement of electrodes with sophisticated neural algorithms to improve the level of accuracy of EEG data that is fed into DL models. In order to improve contextual accuracy, Puka et al. (2023) incorporated psychological elements into deep learning frameworks to correlate seizure predictions with patient-reported symptoms.

## CONCLUSION

A revolutionary method for anticipating epileptic seizures is the amalgamation of algorithms that use deep learning, such as CNNs, RNNs, and ANNs, with optimal EEG channel selection. In comparison to conventional techniques, this approach improves prediction reliability, recollection, and overall accuracy by utilizing the advantages of various neural network topologies to identify either chronological and spatial patterns from EEG data. This method decreases computer complexity and preserves crucial data integrity by identifying the most pertinent EEG channels, enabling real-time seizure prediction. This development highlights the way advanced analysis and optimized channels have the potential to completely transform the treatment of epilepsy and open the door to more affordable and efficient medical treatments.

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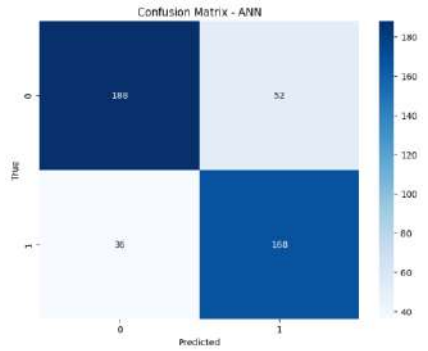
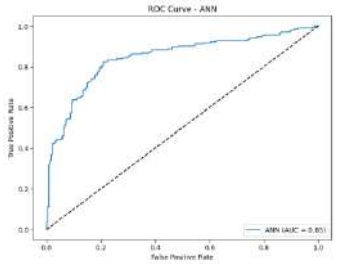
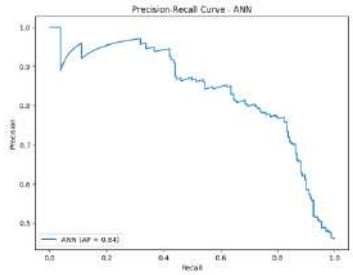
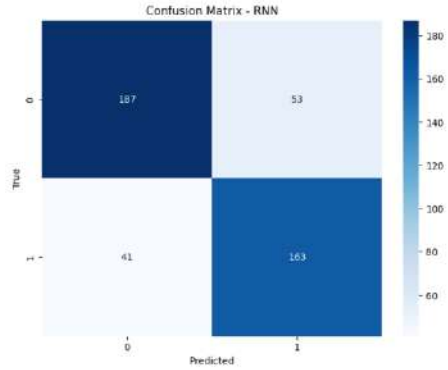
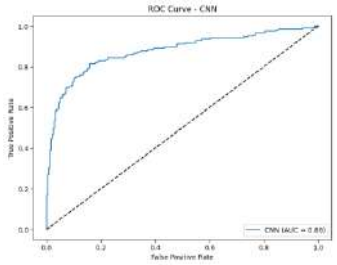
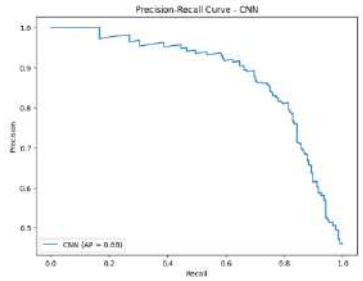
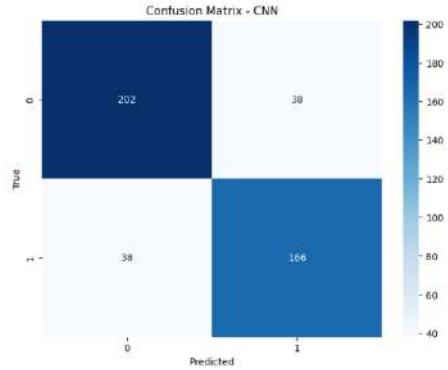
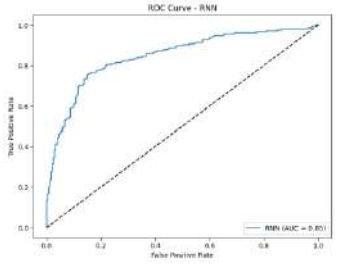
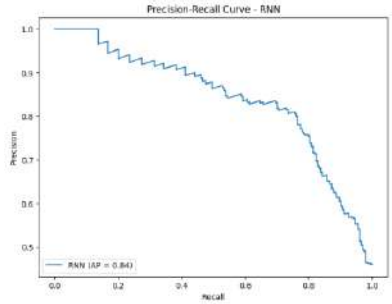
**Table 1: Evaluation Metrics of the Applied Algorithms**

| S.NO | ALGORITHM | ACCURACY | PRECISION | RECALL | F1 SCORE |
|---|---|---|---|---|---|
| 1 | ANN | 0.81 | 0.84 | 0.78 | 0.81 |
| 2 | CNN | 0.82 | 0.84 | 0.84 | 0.84 |
| 3 | RNN | 0.78 | 0.82 | 0.78 | 0.80 |

**Table 2 : The table 2 shows the confusion matrix and roc curve and precision recall curve for all the applied algorithms like ANN, RNN & CNN**

| CONFUSION MATRIX | ROC CURVE | PRECISION RECALL CURVE |
|---|---|---|
|   |   |   |
|   |   |   |
|   |   |   |

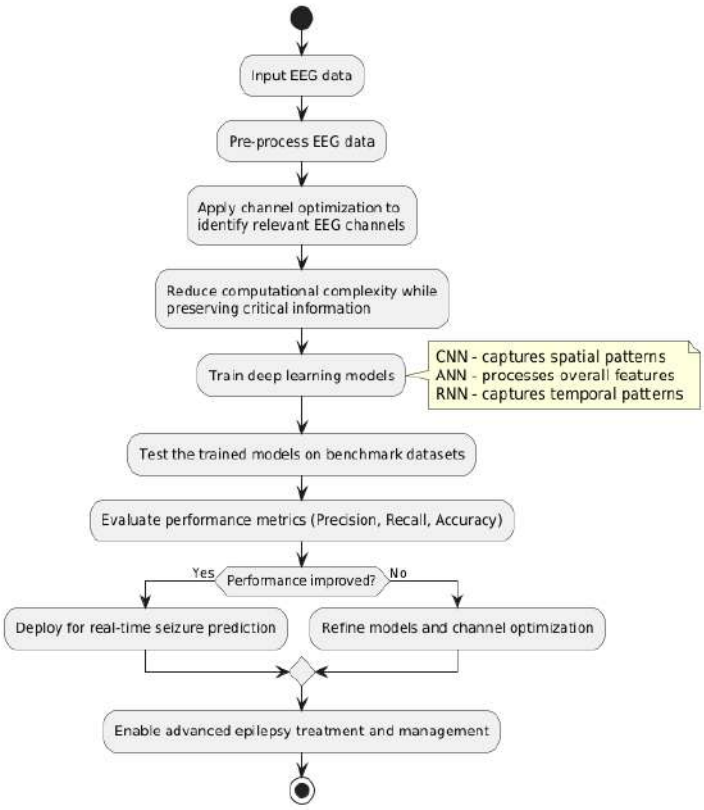



**Fig 1 : The steps from gathering information to the finalized implementation for the prediction of seizures and medical oversight are graphically represented in the schematic above**

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**REVIEW ARTICLE**

# Review on Natural Idempotent Pair in Ternary Semiring

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**ABSTRACT**

In this paper, we study about Natual Idempotent Pair (NIP) in a Ternary semiring. We discussed various characterization of NIP in Ternary semiring. When NIP coincideswith left(right) singular and also investigated the relation between NIP and lateral singular.

**2010 Mathematics Subject Classification:** 16U40,16Y60.

**Keywords :**Ternary Semiring (T.S.R), regular, singular, idempotent, Multiplicative Sub Idempotent (MSI)

## INTRODUCTION

Ternary algebraic system was introduced byD. H. Legmer [6] in 1932. He investigated certain Ternary algebraic systems called triplexes which turn out to be commutative Ternary groups. We have introduced the notion of Ternary semiring which generalizes the notion of Ternary ring introduced by Lister [4] and regular Ternary rings were studied by Vasile in [7], Dutta and Kar [5] introduced the notion of Ternary semiring. By a Ternary semiring we mean an algebraic system consisting of a non-empty set T together with a binary operation, called addtion and Ternary multiplication. Which forms a commutative semigroup relative to addition, a Ternary semigroup relative to multiplication and left, right, lateral distributive laws hold.The set $Z^-$of all negative integers is a natural example of a Ternary semiring. Thus we see that in the ring of integers. Z, $Z^+$forms semiring where as $Z^-$ forms a Ternary

semiring. Our main purpose of this paper is to study the notion of NIP in a Ternary semiring and some their properties.

## Preliminaries

**Definition 2.1**. If $T$ is an additive commutative semigroup that satisfies the following requirements, then a set that is not empty $T$ with a binary addition and a multiplication of ternary structures, indicated by juxtaposition, together form a Ternary Semiring:

1. $stu \in T$,
2. $[stu]vw = s[tuv]w = st[uvw]$,
3. $[s+t]uv = suv + tuv$,
4. $s[t+u]v = stv + suv$,
5. $st[u+v] = stu + stv \ \forall \ s,t,u,v,w \in T$

**Example 2.1.** Let Z− be the set of all negative integers. Then, with the binary addition and Ternary multiplication [ ] defined by $[stu] = stu$ for all$s,t,u \in Z^-$, $Z^-$ forms a Ternary semiring.

**Definition 2.2.** A Ternary semiring T is said to be multiplicatively left cancellative (MLC), (laterlly cancellative (MLLC) and right cancellative (MRC) ) if $stx = sty$, $sxt = syt$ and $xst = yst$ implies that $x = y$, for all $s,t,x,y \in T$

**Definition 2.3.**If $t + t = t(t^3 = t)$, then $t \in T$ is additively idempotent (multiplicatively idempotent).

**Definition 2.4.**If $t + t^3 = t$, then an element $t \in T$ is multiplicative sub idempotent.

**Definition 2.5.**Ternary Semiring $(T,.)$ satisfies the identity $st^2 = s$ $(t^2s = s,\ tst = s)$, it is considered left (right, lateral) singular for all $s,t$ in T. A $(T,.)$ is regarded as singular if it's Lateral, Left, and Right singular. A T.S.R (T,.) is said to be two sided singular, if it is both are left and right singular.

**Definition 2.6**. In $T$, an element '$s$' has become regular (additive regular) if there is an element $t \in T$ such that $s = ststs(s = s + t + s$, with some $t$ being additive 1-inverse of $s$ ).

**Definition 2.7.**If $\exists\ x,y \in T \ni s = s^3xy$ $(s = xys^3,\ s = xs^3y)$, then a Ternary semiRing (T,.) is considered left (right, lateral) regular. A $(T,.)$ is considered Regular whenever it's Lateral, Left, and Right regular

**Definition 2.8.** An element $s$ of a ternary semiring T is said to be intra regular if there exist $x,y \in T$ such that $s = xs^5y$.

**Definition 2.9.** A Ternary semiring (T,.) is said to be two sided regular, if it left as well as right regular.

**Definition 2.10.** A pair $(s,t)$ of elements in a ternary semiring $T$ is called an idempotent pair if $st(stx) = stx$ and $(xst)st = xst$ for all $x \in T$ [1].

**Definition 2.11.** A pair $(s,t)$ is called a Natural Idempotent Pair (NIP) if $sts = s$ and $tst = s$ [1].

**Definition 2.12.** Two idempotent pairs $(s,t)$ and $(u,v)$ are said to be commute if
$st(uvx) = uv(stx)$ and $(xst)uv = (xuv)st$ for all $x \in T$

**Definition 2.13.**If $s^2t = s + t$ (or $st^2 = s + t \ \forall \ s,t \in T$), then (T,+,.) referred as Mono-Ternary Semiring.

**Definition 2.14.**$If\ s + s^3 = s^3$, then an element '$s$' of a T.S.R T is considered almost idempotent. A T.S.R is almost idempotent if all of the elements in T are almost idempotent.

**Definition 2.15.** For any $s$ and $t$ in T, if $s + t = s$ $(s + t = t)$, subsequently a Ternary Semigroup $(T,+)$ becomes a left (right) singular.

## MAIN RESULTS

Let $T$ be a Ternary semiring and Using addition and multiplication cancellation laws we obtained some results.

**Theorem 3.1**. For $s, t \in T$ is multiplicative sub idempotent and NIP

(i) If $sts = s$ then $t + t = t$

(ii) If $tst = t$ then $s + s = s$

**Proof:**

**From (i)**

Since $s, t$ is MSI we have $s + s^3 = s$ *and* $t + t^3 = s$ for all $s, t \in T$

$sts = s$

$s(t+t^3)s = s$

$sts + st^3s = sts$

$s + t^2s = s$

$sts + t^2s = sts$

$s + t = s$ ---------- (A)

From Equation (A) we have $s + t + t = s + t$ using left additive cancellation law we get

$$t + t = t$$

**From (ii)**

Since $s, t$ is MSI we have $s + s^3 = s$ *and* $t + t^3 = s$ for all $s, t \in T$

$tst = t$

$t(s+s^3)t = t$

$tst + ts^3t = tst$

$t + s^2t = t$

$tst + s^2t = tst$

$t + s = t$ ---------- (B)

From Equation (B) we have $t + s + s = t + s$ using left additive cancellation law we get $s + s = s$.

**Lemma 3.1**:For $s, t \in T$ is NIP then $s, t$ is regular.

**Proof:** which is obvious.

**Lemma 3.2:**For $(T, +)$ is left singular then $(T, +)$ is idempotent.

**Proof:** Since $(T, +)$ is left singular we have $t + s = t$

$t + s + t = t$

$t + t = t$

Therefore $(T, +)$ is idempotent.

**Theorem 3.2**:For $(s, t)$ is NIP in $T$ then the following are equivalent

(i) $(T, +)$ is left singular.

(ii) $(T, .)$ is multiplicative sub idempotent.

(iii) $(T, .)$ is Mono Ternary Semiring

**Proof:**

**From (i)⇔(ii)**

Since $(T, +)$ is left singular we have $t + s = t$

$t + sts = t$

$t + s(t + s)s = t$

$t + sts + s^3 = t + s$

$s + s^3 = s$

Therefore $(T,.)$ is multiplicative sub idempotent.
Converse since $(T,.)$ is multiplicative sub idempotent we have $s + s^3 = s \;\; for\ all \;\; s \in T$
$s + sstss = s$
$sts + s^2tss = sts$
$t + sts = t$
$\mathrm{t} + \mathrm{s} = t$
Therefore $(T,+)$ is left singular.

**From (i)⇔(iii)**
Since $T$ is additive left singular and NIP we have $sts + t = s$
$st(s + t) + t = s$
$\mathrm{sts} + st^2 + t = s + t$
$st^2 = s + t$ (from Lemma 2)

Therefore $(T,.)$ is Mono Ternary Semiring.
Converse, since $T$ is Mono Ternary Semiring and NIP we have $sstst = s + t$
$sstst = sst \Rightarrow s^2t = s \Rightarrow s + t = s$
Therefore $(T,+)$ is left singular.

**Lemma 3.3**:For $s \in T$ is MSI then $s$ is additive idempotent.
**Proof:** which is obvious

**Theorem 3.3:**If $T$ is multiplicative sub idempotent then the following are equivalent
(i) NIP.
(ii) $(T,+)$ is left singular.

**Proof:**
**From(i)⇒(ii)**
Since $(s,t)$ is natural idempotent pair and MSI we have $s(t + t^3)\mathrm{s} = s$
$sts + st^3\mathrm{s} = s\mathrm{ts}$
$st(s + t^2s) = sts$
$s + tts = s$
$s + ststts = s$
$sts + tstts = ststs$
$(s + \mathrm{tst})\mathrm{ts} = ststs$
$\mathrm{s} + \mathrm{t} = s$
Therefore $(T,+)$ is left singular.

**From (ii) ⇒(i)**
Since $(T,+)$ is left singular and MSI we have $s + s^3 + \mathrm{t} = s$
$s + \mathrm{s}(\mathrm{s} + \mathrm{t})\mathrm{s} + \mathrm{t} = s$
$s + s^3 + sts + t = s + s + t$ (from Lemma 3)
$\mathrm{sts} = s$
In similar manner we can prove $\mathrm{tst} = t$

**Theorem 3.4**:If (s, t) ∈ T is NIP and MSI then $(T,.)$ is two sided singular.
**Proof :**Since $(s,t)$ is natural idempotent pair and MSI we have $s(t+t^3)s = s$
$sts + st^3s = sts$
$st(s+t^2s) = sts$
$s + tts = s$
$s + tts = s + s$ (from Lemma 3)
$t^2s = s$
Therefore $(T,.)$ is right singular.
Since $(s,t)$ is natural idempotent pair and MSI we have $s(t+t^3)s = s$
$sts + st^3s = sts$
$(s+st^2)ts = sts$
$s + stt = s$
$s + stt = s + s$ (from Lemma 3)
$st^2 = s$
Therefore $(T,.)$ is left singular.

**Remark 3.1**:If $(s,t)$ ∈ T is NIP iff $(T,.)$ lateral singular (when $s = t$ )
**Remark 3.2:**For non equal elements $s,t$ we have only two sided singular not satisfies singular, which is illustrated in the following example.

**Example 1**. Let $i,j,k \in T$, is a Ternary semiring

| + | i | j | k |
|---|---|---|---|
| i | i | j | k |
| j | j | j | k |
| k | k | k | k |

| × | i | j | k |
|---|---|---|---|
| i | i | j | k |
| j | i | k | k |
| k | k | j | k |

We taking $s = k,\ t = j$
$kjk = k \Rightarrow k.j.k \Rightarrow j.k \Rightarrow k$
$jkj = j \Rightarrow j.k.j \Rightarrow k.j \Rightarrow j$
$k^3 = k \Rightarrow k.k.k = k$
$j^3 = j \Rightarrow j.j.j = j$
(i) $st^2 = s.t.t \Rightarrow k.j.j \Rightarrow k$
(ii) $t^2s = t.t.s \Rightarrow j.j.k \Rightarrow k$
(iii)$tst = t.s.t \Rightarrow j.k.j \Rightarrow j$

In Dutta and Kar proved that Idempotent Pair equivalent to NIP for '$s$' is multiple of '$t$' (or) '$t$' is multiple of '$s$'. In this paper we discussed without the multiples and proved the relation between NIP and Idempotent Pair. Hence it easy to verify that '$s$' is binary regular leads to Ternary regular need not the converse
(i.e) $s = sts$
$s = st(sts)$
$s = ststs$

Therefore $s$ is regular in $T$. In [1] they need semi-inverse for Ternary regular in page 348. In the same paper [1] Dutta and Kar examined the Idempotent pair equal to NIP and also regular element equal to Idempotent Pair. '$l$' is Ternary regular and not binary regular.

which is illustrated in the following example.

**Example 2**. Let $l, m, n \in T$ is a Ternary semiring

| + | l | m | n |
|---|---|---|---|
| l | l | m | n |
| m | m | m | n |
| n | n | n | n |

| × | l | m | n |
|---|---|---|---|
| l | l | m | n |
| m | n | n | n |
| n | n | l | n |

We taking $s = l,\ \ t = m$
$lmlml\ =\ l.m.l.m.l$
$= m.l.m.l\ = n.m.l\ = l.l = l$
$lmlml\ =\ l$
$lml\ =\ l.m.l\ = m.l\ = n$
$lml =\ n$
Here $l$ is Ternary regular but not a binary regular.

**Theorem 3.5**: For $(s,t) \in T$ is NIP and $(T,.)$ is idempotent then the following are equivalent

(i) $(T,.)$ is regular
(ii) $(T,.)$ is right regular
(iii) $(T,.)$ is lateral regular
(iv) $(T,.)$ is left regular

**Proof :(i) ⇒ (ii)**
Since T is regular and idempotent we have $stsssts = s$
$st^3s^3ts = sts$
$tts^3 = s$
Therefore (T,.) is right regular
**($ii$) ⇒(iii)**
Since T is right regular and NIP we have $tsttsss = s$
$ts^3tts = sts$
Therefore (T,.) is lateral regular.
**(iii)⇒(iv)**
Since T is lateral regular and idempotent we have $tsssttt = ts^3t$
$tsssttt = tst$
We obtain (T,.) is left regular
**(iv)⇒(i)**
Since T is left regular and NIP we have $ssstt = s$
$ssstststt = ssstt$
Therefore $(T,.)$ is regular.

**Theorem 3.6**: For $(s,t) \in T$ is NIP then the following are equaivalent

(i) $(T,.)$ is idempotent
(ii) $(T,.)$ is two sided singular

**Proof : (i)⇒(ii)**
Since T is NIP and idempotent we have $sttts = s\ implies\ that\ st^2 = s$ and $t^2s = s$ we obtain (T,.) is two sided singular.

**(ii)⇒(i)**
Since $(T,.)$ is two sided singular
Using multiplicative left cancellation law, we obtain
T is multiplicative idempotent.

**Lemma 3.4:**
A Ternary semiring is Natural idempotent pair and additive right singular then a ternary semiring is multiplicative idempotent.
**Proof :** Since s is natural idempotent pair and additive right singular, we have
$s + t = t$
$sts + t = t$
$s(s + t)s + t = t$
$s^3 + sts + t = s + t$
$s^3 + s = s + s$ (from Lemma 2)
Therefore T is multiplicative idempotent.

**Theorem 3.7**: For $(s,t) \in T$ is NIP then the following are equivalent
(i) $T$ is almost idempotent
(ii) T is additive right singular

**Proof : (i)⇒(ii)**
Since $s$ is almost idempotent and natural idempotent pair we have
$s + s(sts)s = sss$
$t + sts = s$
$t + s = s$
Therefore T is additive right singular.
**(ii)⇒(i)**
Since T is additive left singular using NIP we have
$t + s^3 + sts = t + s$
$t + s^3 + s = t + s$
$s^3 + s = s$
$s + s^3 = s^3$ (from lemma 4)
Therefore T is almost idempotent.

**Theorem 3.8:** For $(s,t) \in T$ is NIP and idempotent then
(i) $s$ is left singular iff $t$ is a left singular.
(ii) $s$ is right singular iff $t$ is a right singular.

**Proof : (i)⇒(ii)**
Since $s$ left singular and idempotent we have
$st^2 = sts^3$
$\Rightarrow stst^2 = ststs^3$
$\Rightarrow tst = tstss$
$t = ts^2$
$\therefore t$ is left singular
Converse, let $t$ is left singular and idempotent we have
$ts^2 = ts\,t^3$
$tsts^2 = tstst^3$
$s = ststt$
$s = st^2$
$\therefore s$ is left singular

**(ii)⇒(i)** In similar manner we can prove.

**Theorem 3.9:** A ternary semiring is natural idempotent pair and multiplicatively left (right) singular thena ternary semiring is intra regular.

**Proof :** Let $(s,t)$is NIP and two sided singular in a ternary semiring T then for each $s \in T$ for some $t$ such that $st^2 = s = t^2s$. If we take $sts = s$ there exists $t \in T$ we have $stst^5sts = s$. By using left and right cancellation law, we get $st^5s = t$ this implies that $T$ is intra regular ternary semiring.

**Theorem 3.10**:A pair $(s,t) \in T$ is NIP and $T$ is multiplicatively two sided singular then the following hold:

(i) $t = t^{4n+1}, n \geq 1.$

(ii) $s = st^{4n+1}s, n \geq 1.$

**Proof:**(i) Let $(T,.)$ is right (left) singular and NIP we have $s = st^2tt^2s$

$s = st^5s$

$t = t^5$ ----------(1)

We take equation (1) $t = t.t^4$ then we get $t = t^5.t^4$

$t = t^9$ ----------(2)

In this way to continue we get $t = t^{13}$

In general we have the following $t = t^{4n+1} \forall\, t \in$ T and $n \geq 1$.

(ii) We take $sts = s$

From the equation (1) in NIP we get

$s = st^5s$ ----------(3)

$s = st.t^4s$

$s = st^5.t^4s$

$s = st^9s$ ----------(4)

$s = st^5.t^8s$

$s = st^{13}s$ ----------(5)

In this way to continue we get $s = st^5s = st^9s = st^{13}s \ldots$

In general we have the following $s = st^{4n+1}s \forall\, t \in$ T and $n \geq 1$.

**Theorem 3.11:** If $T$ is MSI then the following are equivalent

(i) $(s,t) \in T$ is NIP

(ii) $s^2t = s = ts^2$

(iii) $t^2s = t = st^2$

**Proof: (i)⇔(ii)**

Let $sts = s$ using MSI we have $sts + s^3ts = s$

$s + s^3ts = s + s^3$

$s^2t = s$

Similarly to proved $ts^2 = s$

Converse, since $s^2t = s$ and MSI we have $sst = s + s^3$

$sst = s + s(s^2t)s$

$sst = s + s^3ts$

$s = s + s^3ts$

$s + s^3 = s + s^3ts$

$$sts = s$$

Similarly, to proved $tst = t$
(i)⇔(iii) In similar manner we can prove.

**Theorem 3.12:** For $(s, t)$ is NIP in a Ternary semiring,

(i) $s$ is idempotent then $tst$ is idempotent
(ii) $t$ is idempotent then $sts$ is idempotent

**Proof.** (i) Let $tst = t$
$ts^3t = t$ ($\because$ $s^3 = s$)
$tstsstsstst = t$ ($\because$ $s \in NIP$ )
$tstttst = t$ ( $from\ theorem\ 6$ )
$tsttsttst = tst$
Therefore $tst$ is idempotent w.r.t.multiplication
(ii) similar manner by (i) we can prove.

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**RESEARCH ARTICLE**

# Empowering Rural Communities with the Integration of IoT and Machine Learning

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**ABSTRACT**

Infrastructure, which took place in health care as well as agriculture may all be significantly improved in rural regions by integrating IoT and machine learning. Agriculturalists are able to track the overall wellness of their livestock, maximize crop production, and effectively administer supplies by utilizing statistical information and linked equipment. Diagnostic tools and distant monitoring improve accessibility to health care amenities. Improved water and electrical management enhances infrastructure management by guaranteeing dependable and long-lasting services. In order to demonstrate the revolutionary effects of contemporary technology on rural communities, this study looks at scenarios and real-world applications. The findings show how contemporary technology may empower and enhance rural communities by highlighting increased profitability, greater quality of life, and sustainable growth.

**Keywords:** contemporary, rural communities, agriculture, IoT,

## INTRODUCTION

The combination of Internet of Things and machine learning can have a major positive impact on rural communities, which are frequently left out of technology progress. By maximizing crop yields along with utilizing resources

efficiently, these advancements have the potential to revolutionize agriculture. They are additionally capable of enhancing healthcare via telephone medical care and monitoring remotely, and they can strengthen transportation by effectively managing water and electricity. Good outcomes have already been observed in several places from practical applications, such as distant medical treatment programmes and precision farming operations. To guarantee fair access, however, issues like affordability, competence with computers, and accessibility need to be resolved. Eliminating these challenges can lead to higher living standards, enhanced efficiency, and improved sustainability in rural areas.

**Technology in Rural development**

Despite being sometimes disregarded in improvement of technology, rural areas have enormous opportunities for progression and growth. These populations can overcome constant problems by using modern innovations including artificial comprehension and the Internet of Things. By utilizing these resources, rural communities can see notable advancements in a number of areas, improving the standard of living and job prospects for locals.

**Advancements in Agriculture**

The Internet of Things and machine learning have enormous potential benefits for agriculture, which is the foundation of many agricultural economies. Agriculture can track the performance of crops, the climate, and dirt conditions in real time with the use of interactive sensors and interconnected devices. These data are analyzed by machine learning techniques, which yield useful information for improving fertilization, irrigation, and pest management. Improved crop yields, lesser resource waste, and more farmer prosperity are the results of these advances.

**Enchanting HealthCare Access**

Access to healthcare is frequently restricted in rural locations; however, IoT and machine learning present creative options. Periodic medical monitoring is made possible by direct equipment for monitoring and telemedicine platforms that provide anonymous interactions with doctors. Through the analysis of patient data, machine learning can help with personalized treatment strategies and early identification. These developments guarantee improved healthcare results and lessen the need for patients to travel great distances in order to receive care.

**Infrastructure Management**

The sustained advancement of rural areas is contingent upon the presence of appropriate connectivity. Both energy and water resources may be instantly monitored and managed by IoT devices. IoT-enabled water conservation systems optimize utilization and cut garbage, while distributed energy systems guarantee dependable electricity delivery. Machine learning algorithms forecast the need for maintenance, averting expensive malfunctions and guaranteeing continuous operations. A more robust and environmentally friendly environment results from these upgrades.

**BackGround Work**

For a considerable time, the technological disparity between agricultural and urban areas has hindered progress[1]. Traditionally, rural areas have not had access to the technology developments that propel economic progress and enhance quality of life. These places are frequently isolated and short of funds. But fresh chances to close this distance are presented by the development of machine learning and the Internet of Things[2,3]. Rural communities are able to boost medical products and services, increase agricultural output, and regulate infrastructure more effectively by utilizing these technologies[4]. Real-time information regarding plant health and temperature can be sent to farms using connected devices throughout agriculture, like observatories, drones, and soil sensors[5]. Systematic resource supervision and tracking made possible by this data results in improved fertilization, cultivation, and insect control. The farmers may make intelligent choices by using artificially intelligent technologies to evaluate any information that these sensors collect and provide useful insights[6]. Growing more food is made easier and more ecologically sound with this strategy, which also lowers waste and operating expenses[7]. The application of the internet of things and machine learning has led to notable advancements in health services in rural areas as well.

Patients with ongoing illnesses or impaired mobility benefit greatly from the continual tracking of their health conditions made possible by portable devices[8]. Ehealth platforms enable doctors to interact with patients without having to travel, offering prompt health recommendations and care[9]. Through individual patient information analysis, machine learning improves patient health and lessens the strain on health care agencies by assisting in diagnosing illnesses early and customized treatment programmes[10,11]. IoT and machine intelligence have the potential to greatly affect another important field, which is overseeing the infrastructure[12]. Water's availability systems for management with IoT capabilities and electrical networks that are smart provide contemporaneously managing resources as well as administration, minimizing waste and guaranteeing timely distribution[13,14]. Using machine learning to drive predictive maintenance, possible problems can be found before they become serious, saving money on repairs and wasted time. By constructing robust infrastructure, this technology can help people in rural areas achieve equitable prosperity[15].

## METHODOLOGY

Through the transformation of multiple fields, machine learning may greatly improve situations in rural areas. It can evaluate enormous volumes of information collected comprising weather predictions, crop photos, and sensor readings to optimize pest management, irrigation, and growing times, increasing agricultural yields while lowering expenses. Using machine learning programmes used in healthcare can analyze information about patients and local health data to forecast epidemics of illnesses and help with rapid identification, resulting in prompt medical attention. Furthermore, technology can enhance schooling by customizing learning materials to meet the needs of every pupil separately through personalized courses. Machine learning assists in forecasting and minimizing breakdowns in machinery, lowering costs for upkeep, and enhancing availability of services through the analysis of environmental data. Together, these developments create an agricultural community that is more healthy and resilient.

Through the ability to monitor and manage resources in real-time, the Internet of Things (IoT) significantly contributes to the improvement of rural circumstances. IoT-enabled equipment Wetter stations, & soil- moisture sensors are examples of connected devices in agriculture that give farmers vital information they need to improve agricultural methods, save water, and boost output. Internet of Things technology within health care makes it possible to track remotely patients, particularly those with long-term illnesses, which expedites medical attention and minimizes requirements for travel. IoT may also improve the oversight of infrastructure by keeping an eye on the state of energy grids, water lines, and roadways to guarantee prompt upkeep and effective resource usage. The Internet of Things (IoT) makes rural living easier and healthier by tying together disparate systems and gadgets.

Combining IoT with machine learning can have a major positive influence on rural communities due to their complementary benefits. IoT devices gather enormous volumes of info through a variety of other sources, including buildings, agricultural land, and healthcare. The collected information is then analyzed by machine learning algorithms to produce findings that may be put to use. IoT sensors, for instance, may constantly track crops and soil conditions throughout food production, and AI models can identify early warning indicators of illness or infestations and forecast the best agricultural techniques. IoT-enabled medical gadgets are able to measure patients' vital signs during actual time. Furthermore, deep learning algorithms offer predictive analytics that enable prompt diagnosis and individualized treatment strategies. IoT-enabled infrastructures may communicate their current state, and algorithmic learning can anticipate problems to allow for on-time replacement.

## RESULTS AND DISCUSSION

**Fig 1:** Prediction of the algorithms in different aspects like weather conditions and yield prediction at various ranges

## CONCLUSION

By tackling major issues and advancing ethical growth, connecting IoT, as well as machines can drastically change rural communities. AI offers data-driven insights to improve education with individualized instruction, optimize the agricultural sector, and improve medicine with timely diagnoses and personalized therapies. IoT makes it possible for companies to track throughout real time along with managing resources efficiently in a variety of industries, including infrastructures and agribusiness. These advances in technology combine to benefit rural areas by building one intelligent networked ecosystem.

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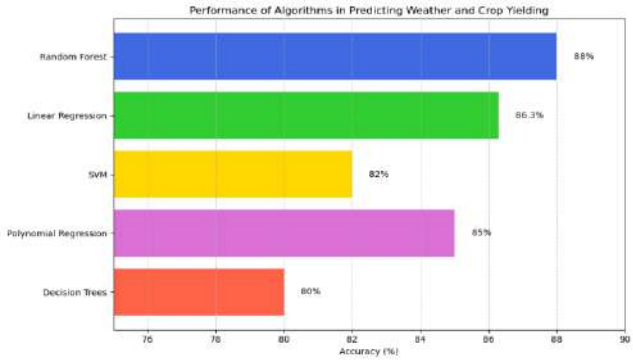


**Fig 1: Prediction of the algorithms in different aspects like weather conditions and yield prediction at various ranges**

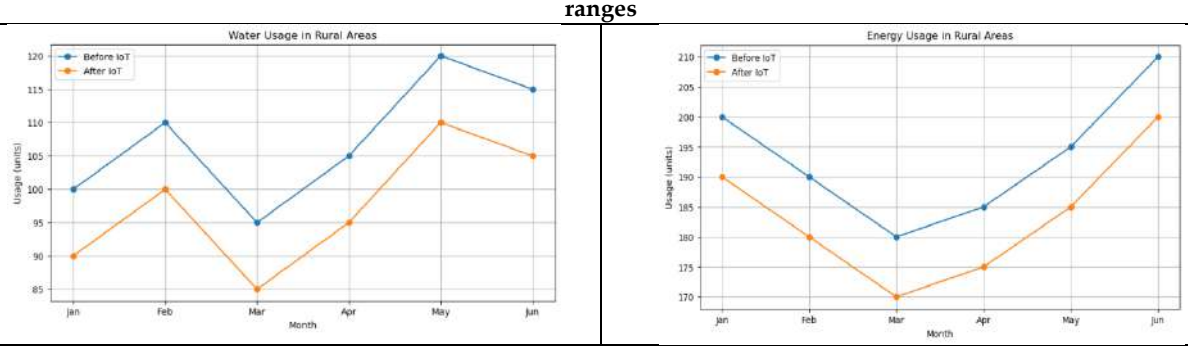


**Fig 2 : Water & Energy Usage**

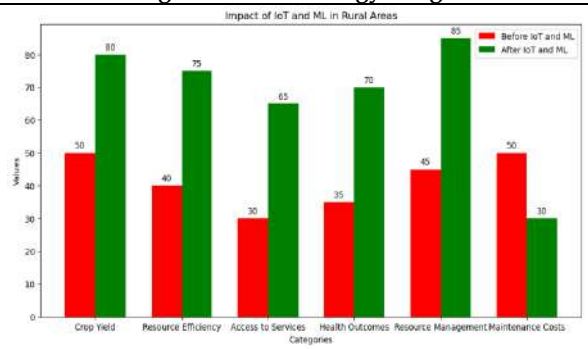


**Fig 3 : Impact of IoT and ML in Rural Areas**

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**REVIEW ARTICLE**

# Controlled Drug Release Delivery Systems: A Comprehensive Review

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## ABSTRACT

Controlled-release systems manage the level of a drug in a patient's plasma after administration by using pre-set parameters. Patterns are established over a specified timeframe. The release rate should dictate drug absorption and concentration levels. These decreasing the frequency of daily doses can be achieved through formulations. This article covers key specifications, benefits, and characteristics. Methods and approaches for developing formulations that release drugs in a controlled manner to enhance drug delivery. This involves administering medication at a predetermined rate for a specific duration, either within a localized area or throughout the entire system. Utilizing drug-encapsulating devices, this method provides benefits over conventional approaches, such as customised release rates and drug delivery. Enhanced patient protection and comfort levels. Controlled-release drug delivery systems sustain a consistent level of plasma concentrations over an extended period. Maintaining a therapeutic concentration level minimizes side effects and reduces the need for frequent administration. Oral Sustained release products modify drug characteristics, decreasing the need for frequent dosing and guaranteeing optimal therapeutic effects. Improved drug efficacy, decreased adverse reactions, and accelerated treatment or management of conditions. Advances in technology have occurred. Transformed medication methods with controlled release systems, providing advantages such as numerous Single and solitary dosing. Oral controlled release drug delivery offers sustained delivery of medications through the mouth at consistent and predictable rates for a controlled period. A predetermined timeframe is focused on particular areas within the gastrointestinal tract in order to achieve local or systemic effects. This method decreases the frequency of drug administration and keeps drug levels consistently stable within the patient. The treatment's impact is augmented by enriching the bloodstream.

**Keywords:** Controlled drug release; Oral formulations; Polymers; Biodegradable polymers; Matrix system.

# INTRODUCTION

Systems for controlled drug release provide advantages like the achievement of targeted drug concentrations. Among the potential drawbacks are toxicity, undesirable by-products, surgery, patient discomfort, and possible side effects [1]. Compared to conventional medications, controlled-release systems have a higher price tag, yet they should possess characteristics such as being motionless, biocompatible, potent, comfortable, secure, simple to administer, and designed to sustain high drug concentrations over a prolonged period. The perfect polymer achieves equilibrium between swelling, erosion and dissolution mechanisms. Achieving high viscosity in the gel state and maintaining a consistent gel layer to facilitate linear drug release over extended periods is a difficult task due to various dynamic phases in polymer relaxation, disentanglement, and erosion [2, 3]. Controlled-release drug delivery systems ensure a steady level of medication in the bloodstream, reducing the risk of adverse reactions and the need for frequent dosing by consistently delivering the drug to the absorption site [4, 5]. The purpose of sustained release systems is to decrease the need for frequent dosing or enhance the potency of a drug by targeting the area where the action occurs, decreasing the amount of medication required, or ensuring consistent delivery of the drug. These systems offer medications over an extended time frame, whereas controlled release systems provide therapeutic regulation. Sustained-release dosage forms are being extensively studied for enhanced patient adherence and reduced misuse. Extensive research in this area has led to multiple breakthroughs, with the unveiling of advanced and highly complex findings. The use of orally administered once-daily products is a cause for concern. This review describes an overview of extended-release products. Theoretical foundation, conventional formulation methods and the current problems and challenges being experienced in the field.

**Controlled release formulations**

The USP defines modified-release formulations as those that utilise the goal of drug release characteristics is to attain therapeutic benefits. Extended-release dosage forms enable the delivery of a two-fold advantage. A reduction in dosing or a substantial increase in a patient's medication may have certain consequences. Most commonly marketed single-piece oral appliances. Most marketed monolithic oral extended. Release dosage forms are categorized into two primary technologies. Hydrophilic, hydrophobic, or inert matrix systems, and coated reservoir systems. These systems incorporate straightforward mechanisms. Diffusion, erosion, or osmotic systems, where the polymer membrane encloses the drug core [6].

**Polymers used in control drug delivery system**

Polymers perform a vital function in the administration of medications, acting as the binders in tablets have a high viscosity. Flow controlling agents are used in liquid mixtures, suspensions, and emulsions comprise various categories [7]. These options can also be available. Utilized as film coatings to conceal the unpleasant taste of drugs and improve their palatability. Modifying release characteristics affects stability. Combining a polymer with a drug is a key aspect of controlled drug delivery (CDD). Device to cause release of the active agent in a drug. Designed beforehand, this approach yields more efficient treatments.

**Characteristics of drugs suitable for controlled release [8-11]**

Exhibit moderate rates of absorption and excretion.
Uniform absorption is achieved throughout the entire gastrointestinal tract.
Administered in doses typically considered small.
Maintain a substantial safety buffer.

**Advantages [12-15]**

Administering a lower dose and less frequently.
Minimizing oscillations is a priority.
Reducing the amount of medication has a diminishing effect on both local and systemic effects.
Effect of fewer drugs minimizes local and systemic difficulty.

Reducing drug accumulation is essential.
Decrease long-term drug use

**Disadvantages [16]**
Sustained release dosage forms (SRDFs) have several disadvantages, like Increased costs and reduced flexibility in dosage options are notable concerns.
Risk of medication leakage, decreased drug uptake.
The potential for delayed action onset and first-pass metabolism exists.
These products also necessitate more expensive manufacturing procedures.
It is not suitable for use with substances that are absorbed by the body at specific times in the GIT.

**Types of controlled drug delivery systems** [17,18]
Controlled drug delivery systems are broadly classified as follows:
1. Oral controlled release system
2. Targeted delivery system
3. Dental systems
4. Ocular systems
5. Transdermal systems
6. Vaginal and uterine systems
7. Injections and implants

**Classification based on mechanism**
The systems used for achieving long-term and regulated drug release can be categorised as follows:

**Systems controlled by diffusion processes**:
The process of diffusion entails the movement of drug molecules. Molecules move from higher to lower concentration according to Fick's law. The drug's release rate is influenced by its diffusion through the membrane barrier [19].

**Reservoir type**
Reservoirs of a similar type to delivery systems are manufactured [19]. This ensures that the medication is released from the delivery system at a gradual pace. In different types of delivery systems, the partitioning of drug molecules within the polymeric membrane is the step that slows down the release process. The controlled release matrix system provides a delivery mechanism. Medications are delivered at a predetermined rate at specified times, with the goal of attaining therapeutic plasma concentrations via optimal delivery patterns [20].

**Dissolution-controlled systems:**
Drugs with high aqueous solubility encounter dissolution rate issues [21]. Dissolution of a substance can be regulated by reducing its rate, encapsulating it within an insoluble polymer, or covering it with polymeric coatings. The rate-limiting step is the diffusion process across the aqueous boundary layer.

**Encapsulation systems**
Microencapsulation procedures use slow-dissolving polymers to coat or encapsulate drug particles [22]. The coating's thickness and solubility dictate its rate of dissolution.

**Matrix systems**
Matrix dissolution systems are a widely employed technique in controlled delivery systems and involve the API being evenly dispersed throughout a polymer matrix. When the polymer matrix breaks down, it usually does so through erosion, allowing drug molecules to be released into the surrounding environment [23].

**Water penetration-controlled systems:**
Control of the system's pressure is achieved through the entry of water into the system [24].

**Systems that regulate swelling**
Systems that release fluids can absorb body fluids, causing them to swell. Increasing the solvent content and the size of the polymer mesh allows for drug diffusion through the swollen networks.

**Osmotic controlled release systems** (OCRS)
OCRS are characterised by every osmotic system incorporating a semi-permeable membrane. The medication delivery method consists of an osmotic core and a semi-permeable membrane, which controls water flow. The device has only one opening, and medication is always dispensed in a liquid form. Effective only with water-soluble medications. The drug release process exhibits a zero-order kinetic profile [25].

**Applications [26]**
Prescription medications that are formulated for gradual release are advantageous for individuals receiving treatment. Patients with long-term conditions like diabetes and high blood pressure suffer from these illnesses. Conditions such as asthma and epilepsy, along with neurological disorders are treated with CDDS. Conditions such as Alzheimer's and Parkinson's disease, hormone therapy, pain management also receiving CDDS. Better control and fewer side effects have been observed. Controlled Contemporary drug delivery systems are applied in numerous fields. Covering fields such as cancer treatment, and ophthalmology. The CDDS offer extended periods of respite, thus decreasing the necessity for repeating dosages should be kept to a minimum to reduce the potential for addiction.

## CONCLUSION

Dosage forms are combined with additives to increase their effectiveness. Stability and flavor are crucial elements. Traditional pill and capsule forms are often ineffective. Due to varying plasma drug concentrations, high dosing is necessary. Controlled drug delivery systems enhance the bioavailability, make possible the controlled release, and minimise side effects while maintaining plasma levels. These systems comprise dissolution, diffusion, water penetration. The delivery is controlled by stimuli and chemical factors. Delivery systems have applications in managing disease conditions. Future drug delivery systems prioritize tailored treatment approaches based on individual patient needs, utilizing. Devices manufactured through microfluidics, three-dimensional printing, and utilising CRISPR cas9 technology. The incorporation of contemporary technologies, such as targeted advertisements, modern technologies, including targeted. New concepts have transformed oral controlled delivery systems. Providing benefits over traditional dosage formats.

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**REVIEW ARTICLE**

# A Peer Review on High Performance Liquid Chromatography

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**ABSTRACT**

One of the most common methods for determining and measuring the effectiveness of pharmaceutical ingredients and drugs is high-performance liquid chromatography. Two very important steps are performed before the method is released for use in the quality control department: analytical method development and validation. A step-by-step practical approach to establishing an RP-HPLC test technique is the main topic of this document. Even a novice chromatographer can create a method with the knowledge of the RP-HPLC method development process and its parameters, thanks to the brief explanation of the many contributing parameters and their influence on the performance of the developed RP-HPLC analytical technique.

**Keywords:** chromatography, RP-HPLC, analytical.

## INTRODUCTION

In modern chemistry, high-performance liquid chromatography (HPLC) is an effective analytical tool. Ideal for locating, quantifying and separating components. Sample dissolved in liquid. HPLC is widely used in the analysis of pharmaceuticals and is highly regarded for its accuracy in quantitative and qualitative evaluation, making it a major advance in analytical chemistry [1]. In high-performance liquid chromatography (HPLC), the sample solution also called the stationary phase, is injected into a porous column. A liquid is then pumped through the column under high pressure (the mobile phase).

Components in the sample exhibit different properties due to: Separating the stationary and mobile phases causes them to move at different rates through the column. This causes elution at different times. This allows for separation. The different behavior of components during partitioning gives HPLC its high accuracy and makes it a reliable method for testing a wide range of samples in fields such as analytical chemistry and pharmaceuticals. [2]

In high performance liquid chromatography, compounds with low affinity for the stationary phase move faster and travel longer distances, while molecules with higher affinity move slower and travel shorter distances. This differential migration allows for effective separation and analysis of sample components. [3] Pharmaceutical analysis benefits greatly from high performance liquid chromatography (HPLC). Drugs, degradation products, synthetic intermediates, and reaction impurities can be effectively isolated and quantified. HPLC is the state-of-the-art analytical technique of choice for the detection, quantification, and separation of a wide variety of liquid-soluble sample components. Its accuracy is crucial for the assessment of drug stability, and for both quantitative and qualitative drug testing. In the field of analytical chemistry, HPLC is a key technique for ensuring the quality and safety of pharmaceutical formulations, as it provides a way to thoroughly characterize pharmaceutical samples.

**Importance of RP-HPLC Method Development**

Separation, identification, and quantification of compounds in complex mixtures are common applications of HPLC. RP-HPLC is one of the most widely used and effective types of HPLC. It can be used in a variety of industries, including agriculture, medicine, and environmental protection. One of the most important steps in the analytical process is the development of an efficient RP-HPLC approach, which affects the accuracy, sensitivity and selectivity of the analysis. Its further development is crucial, as it allows for more accurate and precise results in complex combinations. The pharmaceutical sector places great importance on RP-HPLC in terms of drug discovery, quality control and stability testing (Bhatkar et al. 2020). Finding the ideal parameters of mobile phase composition, column type and detection wavelength are only a few of the many aspects that need to be optimized when developing a RP-HPLC technique. Every developer must have a good knowledge of the physical and chemical properties of the material and the ability to distinguish between stable and unstable states of a substance. Reproducibility, accuracy and precision are key elements of the RP-HPLC process. Given the wide acceptance of the method, the development of RP-HPLC techniques is essential. RP-HPLC is used in the pharmaceutical industry to investigate the stability and decomposition rates of these compounds. When testing food and beverages, RP HPLC is used to identify and quantify chemicals, contaminants, and natural substances. In addition, RP HPLC is used to evaluate the potency and purity of therapeutic ingredients and products. When conducting environmental research, RP HPLC allows the detection and quantification of a wide range of contaminants, including herbicides, pesticides, and toxic metals [5].

**HPLC Principles**

The distribution of analytes between the stationary phase and the mobile phase (eluent), typically within the column packing, is the basis of high performance liquid chromatography (HPLC). The chemical composition of the analytes determines the rate at which they pass through the stationary phase, which is the basis of the separation. This principle allows for the accurate separation and analysis of a wide variety of compounds, making HPLC a fundamental technique in analytical chemistry, especially in the pharmaceutical and chemical industries.

**Classification of HPLC can be done as follows:**

1. HPLC is divided into analytical and preparative categories based on the operational range.
2. The various chromatography techniques include size exclusion chromatography, affinity chromatography, adsorption chromatography, etc.
3. Chiral phase chromatography and ion exchange chromatography are classified based on the separation principle.
4. Isocratic and gradient separation techniques distinguish chromatography based on elution technique.HPLC principle

The distribution of the analyte between a stationary phase and a mobile phase (eluent), usually within the packing material of the column, is the foundation of high-performance liquid chromatography (HPLC). The analyte's chemical makeup determines how quickly it passes through the stationary phase, which serves as the foundation for

the separation. This principle enables precise separation and analysis of diverse compounds, making HPLC a fundamental technique in analytical chemistry, particularly in pharmaceutical and chemical industries.

**Types of HPLC**

The phase system used determines whether the High-Performance Liquid Chromatography (HPLC) method is used for analysis. Analytes are categorised by polarity using normal phase chromatography, often known as normal phase HPLC (NP-HPLC). NP-HPLC uses a non-polar Polar analytes are retained through interaction between a polar stationary phase and a mobile phase. This interaction causes elution time to increase as analyte polarity rises. NP-HPLC is a useful analytical method, especially for characterising compounds with different polarities in a variety of domains like chemistry, pharmaceuticals, and environmental studies. It offers efficient separation and provides information on sample composition based on polarity. [7, 8]

**HPLC has many advantages, including:**

High- resolution, simultaneous analysis, extreme sensitivity, excellent repeatability, limited sample size, mild analysis conditions, and ease of fractionation and purification make High-PerformanceLiquid Chromatography (HPLC) a versatile and advantageous technique in various analytical applications. [11]

**The HPLC technique has the characteristics listed below**

Fast analysis and accurate control over mobile phase flow rates are made possible by High-Pressure Liquid Chromatography (HPLC), which uses small-diameter stainless steel or glass columns with much higher mobile phase pressure. This setup improves resolution and efficiency. making HPLC an effective analytical tool for the quick and precise separation of substances in a variety of applications. [12]

**HPLC Method Development:**

Research, development, and manufacturing of pharmaceuticals depend heavily on the creation and validation of analytical techniques. Pharmaceutical items' identity, potency, purity, and efficacy are guaranteed by these techniques. Approach Development entails taking into account variables such as physicochemical properties (pKa, log P, and solubility) in order to choose the best detection mode, particularly for UV detection. An important part of analytical development is the validation of an HPLC technique for stability indication. In order to guarantee the precision and dependability of the analytical procedure in pharmaceutical quality control, it focusses on separating and measuring the main active component, reaction impurities, synthetic intermediates, and degradants. [13]

**HPLC method development**

The following is a step in HPLC method development Establishing an analytical strategy involves selecting chromatographic settings and understanding the physicochemical properties of drug molecules. The subsequent steps include sample preparation, refining the method, and validating it to ensure accuracy and reliability in pharmaceutical analysis. [14]

**Understanding the Physicochemical Properties of Drug Molecules**

A solid understanding of the physicochemical characteristics of a drug molecule is fundamental when designing an analytical method. Key properties to consider include the molecule's pKa, solubility, polarity, and pH. Polarity, in particular, influences the selection of solvents and the mobile phase. The principle of "like dissolves like" plays a significant role in determining molecular solubility, directly impacting solvent compatibility and selection. It's crucial to ensure that analytes are not only soluble but also stable and do not interact adversely with other components. When developing a High-Performance Liquid Chromatography (HPLC) method, factors like pKa and pH are essential, as they influence solvent selection and overall method performance. In HPLC, optimizing the pH is particularly important for ionizable analytes, as it helps produce sharp and symmetrical peaks, which are critical for achieving low detection limits, minimal variability between injections, and consistent retention times, ensuring accuracy and precision in quantitative analyses【15】.

**Selecting Chromatographic Conditions**

The initial phase of method development involves choosing various parameters, such as the detector, column, and mobile phase, to create preliminary chromatograms for the sample. Typically, reversed-phase separations with a C18 column and UV detection are used. At this stage, the choice between using a gradient or isocratic method comes into play, depending on the separation requirements and the properties of the analytes. Both techniques have their advantages depending on the complexity of the separation needed【16】.

**Developing an Analytical Approach**

The process of developing a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method begins with selecting chromatographic parameters, including the mobile phase, column type, flow rate, and pH of the mobile phase. Through a series of trials, each parameter is fine-tuned and tested against system suitability criteria. Key performance indicators for the method include a tailing factor of less than two, a resolution greater than five, a theoretical plate count of more than 2000, a retention time of over five minutes, and a relative standard deviation (R.S.D.) of less than two percent for analyte peak areas. These criteria ensure the reliability and reproducibility of the method. When analyzing two components simultaneously, the detection wavelength is typically selected at the isobestic point, where both compounds show absorbance. The linearity of the method is then assessed by determining the concentration range that produces a linear response. Once validated, the method is applied to a laboratory sample, and the commercial product is adjusted to match the linearity range for analysis. This systematic approach ensures the method's accuracy, practicality, and robustness for simultaneous analysis【17,18】.

**Optimizing the Method**

Method optimization involves identifying potential weaknesses and improving them through experimental design. This process includes evaluating the method's performance with different sample types, equipment configurations, and environmental factors. The iterative optimization process strengthens the method, ensuring its robustness and adaptability across various analytical conditions, making it suitable for diverse HPLC analyses.

**Validation**

Validation is the structured process of confirming that a method meets specific predefined requirements for its intended use. It involves assessing the method's performance and proving its reliability and consistency in meeting defined criteria. Validation provides comprehensive evidence that the method can consistently deliver accurate results, especially when working with low concentrations or challenging conditions, which is crucial in analytical techniques like High-Performance Liquid Chromatography (HPLC)【19】.

## CONCLUSION

Be In conclusion, reversed-phase high-performance liquid chromatography (RP-HPLC) continues to be a vital analytical technique due to its flexibility, precision, and sensitivity. Its importance spans across various fields, including pharmaceuticals, environmental monitoring, and biochemistry, owing to its ability to analyze a wide range of substances, from small molecules to complex macromolecules. Method development in RP-HPLC is critical for optimizing resolution, sensitivity, and reproducibility, requiring a careful and structured approach in selecting appropriate stationary phases, mobile phases, and detection methods. Advancements in column technology and data processing have further enhanced the performance of RP-HPLC, enabling faster and more efficient analyses Due to its versatility, precision, and sensitivity, reversed-phase high-performance liquid chromatography (RP-HPLC) continues to be a cornerstone analytical technique in various fields. Its importance in pharmaceutical, environmental, and biochemical research is underscored by its ability to handle a wide range of analytes, including both small molecules and large, complex macromolecules. Method development in RP-HPLC is a vital process that focuses on optimizing resolution, sensitivity, and reproducibility, requiring a thorough and systematic approach to select appropriate stationary phases, mobile phases, and detection methods.RP-HPLC's performance is further enhanced by advancements in column technologies and data analysis tools, which allow for faster and more reliable separations.

By understanding and optimizing the key parameters during method development, researchers can tailor RP-HPLC to meet specific analytical needs, ensuring consistent and high-quality results. As new innovations in instrumentation and techniques emerge, RP-HPLC remains a robust and essential tool in addressing the growing analytical demands in modern research.

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**RESEARCH ARTICLE**

# Design and *In vitro* Characterization of Extended Release Tablets of Anti Epileptic Drug

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## ABSTRACT

The objective of the present study was to formulate and develop extended release drug delivery system of anti-epileptic drug Divalproex sodium. Preliminary studies with different polymers such as HPMC K100M, PVP K90, Cassava starch were performed. Extended release tablets were prepared by wet granulation method. The prepared granules were evaluated for post compression parameters like bulk and tapped density,CI & hausner's index. The prepared tablets were evaluated for various quality control tests like hardness, friability, average weight and drug content. All the formulations were subjected to in vitro dissolution studies for 12 hours. The drug content wasfound to be between 95.42 ± 0.38% to 99.42 ± 0.26%. The hardness was found to be from 4.73 ± 0.42 to 8.40 ± 0.002 kg/cm$^2$ and all the cases the friability was less than 1%. The *in-vitro* release from the tablets (uncoated & enteric coated) was found sustained over 12 hours with Higuchi kinetics of drug release and release pattern followed anomalous (Non-Fickian) diffusion. The Fourier transform Infrared spectroscopy analyses indicated that there was absence of chemical interaction between the drug and the excipients. The dissolution profiles of the developed formulation and the commercial tablet formulation, The released profile of tablet containing cassava starch showed same drug release profile as that of HPMC K100M.

**Keywords:** Divalproex sodium, Hydroxy propyl methyl Cellulose, Cassava Starch, wet granulation method.

## INTRODUCTION

Epilepsy is a neurological disorder comprising various conditions characterized by recurrent epileptic seizures. These seizures can range from brief and almost unnoticeable episodes to prolonged periods of intense shaking. In epilepsy, seizures often occur without an identifiable immediate cause. However, seizures triggered by a specific underlying cause are not classified as epilepsy. One of the strategies for improving the treatment of epilepsy involves the use of extended-release formulations. These formulations are designed to release the drug slowly over a prolonged period, thereby maintaining consistent therapeutic levels in the bloodstream and optimizing both the efficacy and safety of the medication. Extended-release formulations are beneficial because they help to prevent the extreme fluctuations in drug concentration that can lead to adverse effects, improving both patient convenience and medication adherence. Ideally, an extended-release drug delivery system should provide a constant, zero-order release rate, ensuring stable plasma drug levels over time.Valproic acid, often administered as its sodium salt forms such as divalproex sodium, is an anticonvulsant used in the treatment of epilepsy. The precise mechanisms behind its anticonvulsant effects are not fully understood. Valproic acid may increase the levels of gamma-aminobutyric acid (GABA) in the brain or affect voltage-dependent sodium channels. Valproic acid is also known to function as a histone deacetylase inhibitor and is being studied for potential use in treating HIV and certain types of cancer.

## MATERIALS AND METHODS

The materials used in this study include HPMC K100M, PVP K 90, and Cassava starch, all of which were sourced from SD Fine Chemicals. All chemicals and reagents used in the preparation were of analytical grade.

**Fourier Transform Infrared (FT-IR) Spectroscopy**

To assess the compatibility between the drug (Divalproex sodium) and the excipients (HPMC K 100, Cassava starch, and polyvinylpyrrolidone), FT-IR spectroscopy was employed. This technique helps determine any potential interactions between the drug and the polymers, which could affect the drug's release characteristics in the final formulation. In preparation for the FT-IR analysis, 10 mg of the drug and 400 mg of potassium bromide (KBr) were mixed thoroughly in a mortar. A small amount of this triturated mixture was placed into a pellet maker and compressed under 10 kg/cm² pressure using a hydraulic press. The resulting pellet was then positioned on the sample holder of a Shimadzu FT-IR spectrophotometer. The sample was scanned over a range of 4000 $cm^{-1}$ to 400 $cm^{-1}$ to obtain the FT-IR spectra. The spectra of the individual components (Valproate sodium, HPMC K 100, Cassava starch, and polyvinylpyrrolidone), as well as their physical mixtures, were obtained. These spectra were then analyzed and compared to identify any significant changes or shifts in the functional group peaks, which could indicate potential interactions between the drug and the excipients. Through this method, the study aims to ensure the compatibility of the selected excipients with the drug, which is critical for the development of an effective extended-release formulation.

**Preparation of Divalproex Sodium Tablets**

**Making Tablets of Divalproex Sodium**

**Making Granules**

The wet granulation method was used to create the granules for the Divalproex sodium tablets. According to the formulation, the necessary amounts of hydroxypropyl methylcellulose (HPMC K100), cassava starch, polyvinyl pyrrolidone (PVP K30), valproate sodium, and Avicel PH 102 were weighed (see Table 1). After being moved into a mortar and pestle, these ingredients were thoroughly combined. To make the mixture sluggy, 5% starch paste was added. To create granules, this mass was subsequently run through a sieve with a mesh size of 12. Before being stored for additional processing, the granules were dried for four hours at 50°C and then sieved through sieves no. 22 and no. 44 to guarantee uniform particle size. Talc and magnesium stearate

**Hardness Test**:Tablet requires a certain amount of strength or hardness and resistance to friability to withstand mechanical shakes of handling in manufacture, packaging and shipping. Hardness generally measures the tablet crushing strength. Change in hardness results in differences in disintegration and dissolution characteristics. The crushing strength of the tablet was determined using Monsetto hardness tester.

**Friability Test**: 10 tablets from each batch were tested for friability using a Roche Friabilator, the equipment was operated for 4 minutes at 25 revolutions per minute. The tablets were removed, dusted, re-weighed and the percentage of friability was calculated. Tablets with less than 0.1-0.3% loss in tablet weight were considered acceptable.

Test: Accurately weigh and place 100 mg equivalent of sodium valproate (pre-crushed tablet powder) into a 100 ml volumetric flask, add 70 ml of methanol, sonicate for 60 minutes and make up to 100 ml with methanol. Add 5 ml of the above solution to a 50 ml volumetric flask and top up with diluent (pH 2.0 buffer). In vitro drug release studies [11, 12] . USP Type I dissolution apparatus was used to study the in vitro drug release from various prepared formulations. 900 ml of acidic buffer solution of pH 1.2 for 2 h and phosphate buffer solution of pH 6.8 for 10 h were used as dissolution media. Tablets were kept in cages. Temperature was maintained at 37°C ± 0.5°C and stirring speed was 100 rpm. Samples were taken at regular intervals and the same amount was replaced with fresh dissolution medium. The samples were measured using UV spectrophotometer at 283.5 nm (pH 1.2) and 214 nm (pH 6.8) against blank samples. The release studies were performed in triplicate and the average values were plotted against time.

**Kinetic Modeling of Drug Dissolution Profile:**

The dissolution profile of the most satisfactory formulation was fitted to zero order, first order and Higuchi models to ensure kinetic modelling of drug release. These methods were adopted to determine the most appropriate model.

1. Cumulative percentage of drug released versus time (zero-order kinetics model)
2. Log cumulative percentage of drug remaining versus time (first-order kinetics model)
3. Cumulative percentage of drug released versus square root of time (Higuchi model).

a) Zero-order: Many modified release dosage forms, especially sustained or controlled release dosage forms (dosage forms that release drugs in a planned, predictable, and slower-than-normal manner), have zero-order kinetics. A plot of the cumulative percentage of drugs released versus time is linear.

b) First order: Most conventional dosage forms have this dissolution mechanism. Some modified release formulations, especially sustained release formulations, follow this dissolution pattern. During the dissolution process, the drug molecules are thought to diffuse through a gel-like layer that forms around the drug. A plot of the log cumulative percentage of drug remaining versus time is linear.

c) Higuchi model: Many modified release dosage forms contain some type of matrix system. In such cases, the drug dissolves from the matrix. The dissolution pattern of the drug is determined by the rate of water penetration (diffusion controlled). In the Higuchi model, a plot of cumulative percentage of drug released versus square root of time is a straight line.

## RESULTS AND DISCUSSION

Characteristics of Divalproex Sodium Tablets

Pre-Compression Parameters

Divalproex sodium granules were manufactured by wet granulation method. The granules were evaluated for yield, granule particle size, angle of repose, bulk density, tapped density, Hausner ratio and compressibility index. The yields ranged from 86.13 to 97.81%. The particle size of the granules was between 0.496 ± 0.05 mm and 0.558 ± 0.12 mm. The bulk density of the granules was between 0.305 ± 0.03 and 0.417 ± 0.03 g/ml. The angle of repose varied between 25.45 ± 0.12 and 30.77 ± 0.26. The tapping density was between 0.314 ± 0.04 and 0.471 ± 0.05 g/ml. The

Hausner ratios ranged from 1.056 ± 0.04 to 1.128 ± 0.07, and the compressibility index ranged from 5.28 ± 0.16 to 11.44 ± 0.12.

**Post-compression parameters**

Divalproex sodium tablets were prepared by wet granulation. The results of physicochemical evaluation of the prepared tablets are shown in Table 2. The average weight, hardness, friability, and active ingredient content of the tablets were evaluated. The active ingredient content ranged from 95.42 ± 0.38% to 99.42 ± 0.26%. The hardness ranged from 4.73 ± 0.42 to 8.40 ± 0.002 kg/cm2 and friability was less than 1% in all cases.

***In Vitro* Drug Release Studies**

The n-vitro dissolution studies were carried out on the prepared tablets using USP Type I apparatus. The cumulative percentage release of divalproex sodium from the prepared tablets varied between 64.02 ± 0.42% and 99.32 ± 0.16% at 12 hours depending on the drug polymer ratio.

**Release Kinetics of Enteric Coated Tablets**

The data obtained from the n-vitro release studies of CAP coated tablets (ECF3), Eudragit L100 coated tablets (ECF9) and Drug Coat L100 coated tablets (ECF15) were fitted to various kinetic equations such as: B. Zero order, First order, Higuchi model and Korsmeyer-Peppas model.

## CONCLUSION

Divalproex sodium is an anticonvulsant fatty acid used to treat epilepsy. Its mechanism of therapeutic action is not yet well understood. It may function by increasing gamma-aminobutyric acid levels in the brain or by altering the properties of voltage-gated sodium channels. The stability of divalproex sodium is a function of pH, degrading rapidly in the acidic environment of the stomach but showing acceptable stability under alkaline conditions. Therefore, divalproex sodium needs to be delivered in the intestine. Therefore, attempts have been made to formulate sustained release drug delivery systems for divalproex sodium using various enteric coating polymers. The main objective of the study was to develop sustained release divalproex sodium tablets. The study led to the following conclusions:

The drug divalproex sodium was selected for the study due to its availability, proven efficacy and better clinical use. The divalproex sodium tablets were prepared by wet granulation. The physicochemical results of the manufactured tablets were within the pharmacopoeia limits. Effect of enteric coating on in vitro drug release: None of the enteric coated CAP tablets showed drug release in the first 2 hours at pH 1.2. The formulations coated with Drug Coat L100 and Eudragit L100 showed 0.5% to 1% drug release in the first 2 hours at pH 1.2. The drug release from the tablets was first order diffusion controlled as indicated by higher r2 values in the first order kinetics model and Higuchi model. The n value of the Kolsmeyer-Peppas equation suggested that the release mechanism was supercase II transport. The ECF3 tablet formulation coated with divalproex sodium showed antiepileptic activity. Based on the observations, it can be concluded that the extended-release tablets formulated with divalproex sodium using commonly accepted and physiologically safe polymers and other excipients could show 12-hour extended-release characteristics. The enteric-coated tablets, especially the CAP-coated tablets, did not release the drug over 2 hours at acidic pH 1.2. Thus, they could reduce the dose absorption, prevent the drug degradation at acidic pH 1.2, minimize the blood concentration fluctuation, dose-dependent side effects and cost, and ultimately improve the patient compliance and drug efficacy.

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**Table-1.Formulation composition**

| Batch code | Divalproex sodium | HPMC K 100 | Cassava Starch | PVP K 30 | Avicel PH102 | Starch paste | Talc | Mg. stearate |
|---|---|---|---|---|---|---|---|---|
| F1 | 250 | - | | - | 144 | q.s | 2 | 4 |
| F2 | 250 | 20 | - | - | 124 | q.s | 2 | 4 |
| F3 | 250 | 40 | - | - | 104 | q.s | 2 | 4 |
| F4 | 250 | 60 | - | - | 84 | q.s | 2 | 4 |
| F5 | 250 | 80 | - | - | 64 | q.s | 2 | 4 |
| F6 | 250 | 100 | - | - | 44 | q.s | 2 | 4 |
| F7 | 250 | - | 20 | - | 124 | q.s | 2 | 4 |
| F8 | 250 | - | 40 | - | 104 | q.s | 2 | 4 |
| F9 | 250 | - | 60 | - | 84 | q.s | 2 | 4 |
| F10 | 250 | - | 80 | - | 64 | q.s | 2 | 4 |
| F11 | 250 | - | 100 | - | 44 | q.s | 2 | 4 |
| F12 | 250 | - | - | 20 | 124 | q.s | 2 | 4 |
| F13 | 250 | - | - | 40 | 104 | q.s | 2 | 4 |
| F14 | 250 | - | - | 60 | 84 | q.s | 2 | 4 |
| F15 | 250 | - | - | 80 | 64 | q.s | 2 | 4 |
| F16 | 250 | - | - | 100 | 44 | q.s | 2 | 4 |

**Table-2.Post compression parameters:**

| BatchCode | Parameter | | | |
|---|---|---|---|---|
| | Hardness(kg/cm$^2$)* | Friability(%)** | Average weight (g)** | Drug content(%)*** |
| **F1** | 8.40 ± 0.02 | 0.011 ± 0.021 | 0.501 ± 0.02 | 98.85 ± 0.21 |
| **F2** | 5.80 ± 0.12 | 0.012 ± 0.015 | 0.499 ± 0.12 | 97.71 ± 0.15 |
| **F3** | 6.20 ± 0.35 | 0.016 ± 0.025 | 0.504 ± 0.009 | 98.85 ± 0.34 |
| **F4** | 4.90 ± 0.21 | 0.005 ± 0.034 | 0.503 ± 0.024 | 97.42 ± 0.42 |

| | | | | |
|---|---|---|---|---|
| **F5** | 4.93 ± 0.15 | 0.023 ± 0.015 | 0.508 ± 0.031 | 96.85 ± 0.16 |
| **F6** | 4.73 ± 0.42 | 0.024 ± 0.017 | 0.505 ± 0.015 | 97.14 ± 0.09 |
| **F7** | 5.66 ± 0.17 | 0.240 ± 0.026 | 0.499 ± 0.019 | 98.55 ± 0.48 |
| **F8** | 8.20 ± 0.16 | 0.017 ± 0.035 | 0.509 ± 0.008 | 99.42 ± 0.26 |
| **F9** | 5.60 ± 0.24 | 0.110 ± 0.018 | 0.498 ± 0.007 | 96.85 ± 0.35 |
| **F10** | 5.73 ± 0.25 | 0.110 ± 0.016 | 0.503 ± 0.014 | 96.28 ± 0.42 |
| **F11** | 5.12 ± 0.34 | 0.090 ± 0.026 | 0.506 ± 0.016 | 95.78 ± 0.13 |
| **F12** | 8.06 ± 0.18 | 0.011 ± 0.034 | 0.498 ± 0.027 | 94.57 ± 0.18 |
| **F13** | 7.66 ± 0.09 | 0.019 ± 0.029 | 0.407 ± 0.034 | 95.42 ± 0.38 |
| **F14** | 5.56 ± 0.24 | 0.051 ± 0.017 | 0.406 ± 0.016 | 95.71 ± 0.27 |
| **F15** | 5.83 ± 0.08 | 0.032 ± 0.014 | 0.504 ± 0.006 | 95.71 ± 0.36 |
| **F16** | 6.21 ± 0.13 | 0.023 ± 0.013 | 0.499 ± 0.018 | 96.01 ± 0.15 |

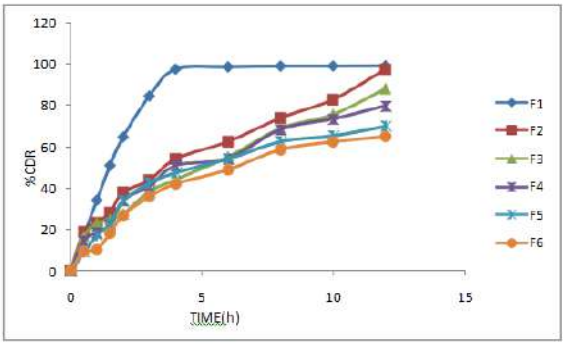


**Figure-1.Dissolution Profile of F1-F6**

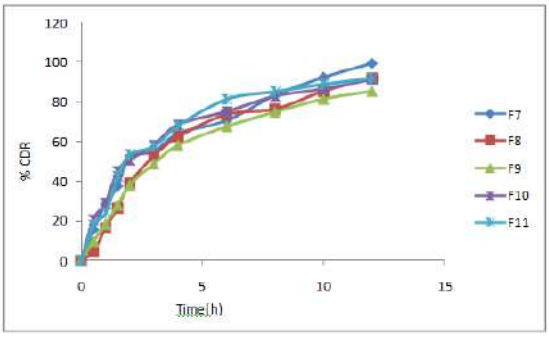


**Figure-2.Dissolution Profile of F7-F11**

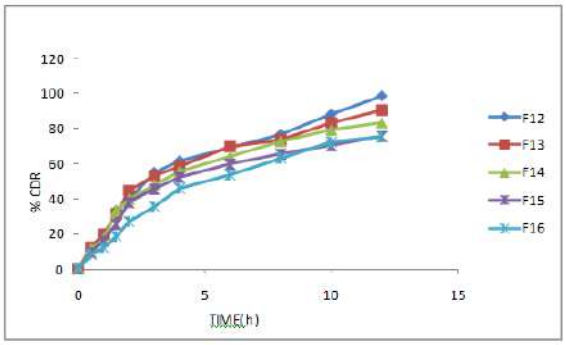


**Figure-3.Dissolution Profile of F12-F16**

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**RESEARCH ARTICLE**

# Enhancing Block Chain Security with Ml - An Intelligent Approach to Fraud Detection and Consensus

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**ABSTRACT**

Despite its inherent security, blockchain systems have drawbacks such as ineffective consensus processes and fraudulent transactions. In order to strengthen blockchain security, the present research incorporates machine learning (ML) approaches, emphasizing fraud detection and consensus process optimization. While Support Vector Machine (SVM) effectively distinguishes between fraudulent and genuine transactions, Random Forest (RF) detects transaction anomalies with high accuracy. Sequential transaction patterns are analyzed by Recurrent Neural Networks (RNN, especially Long Short-Term Memory (LSTM), to detect fraud in real time. Utilizing these methods increases the accuracy of recognition, reduces false alarms, and strengthens consensus processes' dependability. The findings show the proposed ML-enhanced framework offers a strong solution enabling distributed technologies by considerably enhancing blockchain technology efficiency and safety. The advancement of security digital currencies is advanced by integrating it, which guarantees scalability and reliability.

**Key Words** : Algorithms, BlockChain, Machine Learning, Security.

## INTRODUCTION

The exchange of information across the decision-making procedure, machine learning model, blockchain technology node, and application. It describes how to start a transaction, check it to identify deceit, and then use the consensus process to confirm it if there is no fraud. Blockchain technology is a key component for use in supply chain management, healthcare, finance, and other fields because of its independent, open with one another and

trustworthy architecture, which has transformed digital systems. Blockchain systems have serious security issues, including double-spending, fraudulent transactions, and weaknesses in consensus processes, notwithstanding their advantages. In addition to undermining trust, these problems make blockchain networks less scalable and effective. Creative strategies that go beyond conventional cryptography approaches are needed for dealing with these issues.
In order to stop weaknesses in blockchain systems from being exploited, fraud detection is essential. For example, hostile actors, infiltrated nodes, or customer cooperation can all result in illicit transactions. Based on past data, Random Forest is especially good at classification connections and accurately flagging suspicious activity. Similar to this, Support Vector Machine reduces fraudulent results by minimizing judgement restrictions, which helps it distinguish between genuine and unauthorized negotiations. When it comes to modeling temporal dependencies in blockchain transactions, recurrent neural networks (RNN), particularly Long Short-Term Memory (LSTM) networks, provide a distinct edge. RNNs can identify patterns that vary over time, like recurring attempted attacks or slow modifications in transaction behavior, because blockchain data is sequential. Preventative surveillance and immediate form fraud detection depend on this kind of ability. Another crucial component of blockchain performance and security is consensus processes. Even though defined procedures like evidence of function particularly Proof-of-Stake offer strong consensus, they frequently have flaws and inefficiencies when dealing with large transaction volumes or hostile environments. These protocols can identify bottlenecks, eliminate potential attacks, and dynamically adjust to network conditions by incorporating machine learning techniques. This improves overall efficiency and dependability.

## LITERATURE SURVEY

Hasan et al. (2023): To identify fraudulent transactions with credit cards in blockchain-based systems, the authors suggested an improved Random Forest (RF) classifier designed for unbalanced datasets. By employing strategies like the Synthetic Minority Oversampling Technique (SMOTE) to correct class imbalances, their model showed exceptional scalability and accuracy. Because of the increased precision brought about by this method, the RF algorithm is especially well-suited for substantial transactional technologies upon which fraud is uncommon but significant.

Ali and associates (2023) This study created a hybrid framework to detect and lessen fraudulent activity in digital currency transactions by fusing deep learning and Random Forest approaches. Computational efficiency was given top priority in their model, guaranteeing the capacity to handle large transaction volumes instantly. The system's ability to withstand new attack patterns greatly improved blockchain security. Recurrent neural networks (RNNs) were included into the blockchain relies on medical networks by Liu et al. (2021) in order to identify fraudulent data insertion. Their model was highly accurate in detecting irregularities in medical records, protecting individual patient information integrity and enhancing blockchain technology networks' dependability for applications for healthcare.

With an emphasis on developing an approach that not only efficiently indicates fraud but also maximizes consensus, this study investigates the potential for collaboration between machine learning and blockchain technology. According to experimental results, the suggested integration speeds up consensus validation, reduces false alarms, and increases detection accuracy. Additionally, scalability is guaranteed by the use of sophisticated algorithms, which makes the solution suitable for a variety of blockchain applications. Building safe, effective, and scalable systems is made possible by the convergence of blockchain technology and machine learning. The goal of this research is to aid in the expanding field of encryption technology and promote its adoption across businesses by tackling important issues in consensus optimization and fraud detection. The suggested model is a step in the direction of reliable and robust distributed networks. Aburbeian and associates (2023) The authors achieved great accuracy and scalability by concentrating on using Random Forest towards identifying fraudulent credit card transactions in blockchain systems. Additionally, they investigated how well the algorithm handled big, unbalanced datasets, which made it a viable option for actual financial app development. Alghamdi and associates (2022) In

order to identify fraudulent activity in blockchain technology, this systematic study examined machine learning techniques like Support Vector Machines (SVMs) along with artificial neural network models (ANNs). The authors emphasized how well these techniques work to identify complicated patterns and irregularities in autonomous ecosystems. Kumar and associates (2020) Using deep learning models, the research study suggested a blockchain-integrated counterfeiting detection system. The system placed a strong emphasis on improving transaction validation procedures' transparency, minimizing false positives, and detecting unexpected events in immediate aftermath.
Chen and associates (2022) The scientists created a combination of methods to maximize blockchain protocols for consensus by fusing SVMs and RNNs. This method ensured effective network of connections resource use while increasing the accuracy of identifying criminal transactions.

Singh et al. (2021) To prevent fraudulent nodes, the authors devised a prediction model that made use of blockchain technology plus Random Forest. Their system's emphasis on computing efficiency showed how well it preserved the authenticity of distributed systems. Garg et al. (2021) This study investigated the use of RNNs and other cutting-edge machine learning techniques to avoid fraud in blockchain systems. By identifying and addressing irregularities in real-time transactions, the model guaranteed data integrity. Rahman et al. (2022) By employing RNNs to develop an agreement improvement technique, the researchers considerably decreased blockchain vulnerabilities for smart contracts. The way they worked increased the network's resistance against complex assault techniques. Nguyen et al. (2023) To find anomalies in blockchain economic books of account, this study used anomaly detection algorithms like SVM. The technique's exceptional specificity as well as sensitivity make it useful for detecting securities fraud To maximize blockchain security, Sharma et al. (2020) used machine learning models, such as Random Forest. By fixing communication flaws connecting nodes, their efforts improved the safety and effectiveness of the network as a whole.

Gupta et al. (2022) developed a hybrid approach that combines SVM and RNN models to identify deception in blockchain applications enabled by the Internet of Things. Their system showed notable gains in accuracy and dependability, which qualified it for practical use. Patel et al. (2021) improved identifying fraudulent activity in chain of custody systems by combining Random Forest using blockchain-based algorithms. Finding anomalies in the source of information and maintaining supply chain accountability were the main goals of the study. Zhang et al. (2022) developed a hybrid approach for detecting illicit activities in blockchain networks. that makes use of SVM. Their efforts showed improved efficiency along with prevalence of detection while working with big activity databases Representation shows how to improve blockchain security from beginning to end, beginning with loading and preparing the dataset. It combines predictions from models into the blockchain's role in the agreement system for fraud detection and draws attention to the method of selection process (Random Forest, SVM, or RNN)

**Dataset & Preprocessing**

Standard chain databases contain dates and times, individual information about them, historical transaction information, and marks designating reputable or phony transactions. Data Cleaning: Average or maximum extrapolation is used to fix incomplete or contradictory information. Feature analysis is the process of extracting important transactional data including quantity, frequency range, especially user behavior.

Standardization: In order to avoid greater numerical values from dominating, attributes typically balanced.
Encoding: Categorical information (e.g., transaction type) are turned into numerical information using methods of encryption like encoding with one-hot.

**Data Splitting**

Usually in a 70:15:15 ratio, the dataset is separated into sets for training, testing, and validation. The set of experiments assesses how well the finished model performs on unknown data, the set of validation results maximizes parameters, together with the models constructed using data from training.
Dataset Shape: (555719, 23)

**Random Forest's function**

To find important characteristics and spot fraudulent activity

Random Forest is used, Feature Importance Analysis: By assessing each feature's significance, RF streamlines models and enhances interpretability.

Deception recognition: RF creates several decision trees, each of which is trained on an alternate portion of the data. The aforementioned variables trees' estimates are combined using voting by majority to determine if the deal is fictitious or legitimate.

Prediction=Mode(T1,T2,…,Tn)

**Support Vector Machine's (SVM) function**

For accurate fraudulent categorization in data from blockchain platforms, SVM is essential:

The kernel Effects: To separate overlapping classes, SVM uses kernel functions such as the radius basis function (RBF) to translate data into higher dimensions.

Abnormal evaluation: SVM finds anomalies and categorizes them as possible cases of fraud.

$f(x)=sign(i=1\sum n\alpha iyiK(x,xi)+b)$

**Recurrent neural networks' (RNNs') function**

Blockchain technology operations' temporal relationships are modeled by RNNs, especially LSTMs.

Behavioral Novelty Examination: RNNs analyze transaction sequences and spot reoccurring patterns that could be signs of fraud.

LSTMs are capable of detecting minor, dependent upon time deviations that linear models could overlook. This is known to be dynamic identification of anomalies.

$ht=f(Wh\cdot xt+Uh\cdot ht-1+bh)$

**Developing and Assessing Models**

Improved parameter combinations are used to train the computational models. Reliability is evaluated using metrics like ROC-AUC, F1-score, recall, accuracy, and precision. Validation by cross- reduces overfitting and guarantees resilience. For improved prediction performance, ensemble approaches that combine the outputs of RF, SVM, and RNN are investigated.

**BlockChain Technology System Implementation**

The process combines blockchain technology consensus methods like Quantification of Work (PoW) and Proof of Stake (PoS) with machine learning (ML) outputs. Convergence efficiency guarantees effective resource consumption and making decisions in dispersed relationships, while machine learning-enhanced identification of fraudulent activity stops illegitimate operations. By enabling continuous reactions to changing threats and promoting confidence in autonomous systems, this approach fortifies the safety of blockchain networks.

Delivers an explanation of the computerized learning framework's functionality. It specifies classes like BlockchainSecuritySystem for incorporating ML recommendations into blockchain activities, RandomForest, SVM, and RNN for model-specific amenities, including BlockchainData for managing information.

**EXPERIMENTAL ANALYSIS**

Table 1: Performance of the algorithms on the applied data with shape of [Dataset Shape: (555719, 23)]

The Above Graph Shows the Fraud Detection Using the Applied Algorithms

## CONCLUSION

It has been demonstrated that incorporating machine learning algorithms like Random Forest, SVM, and RNN into blockchain security systems is a successful strategy for boosting consensus processes and fraud detection. These models are able to detect fraudulent actions more precisely and effectively than conventional methods by utilizing sophisticated fraud detection strategies. While RNNs, particularly LSTM networks, are good at capturing the sequential relationships found in blockchain transactions, Random Forest and SVM are better at selecting and categorizing features tasks. In addition to improving blockchain network security, the integration of all of these machine learning mathematical models maximizes computational performance by using better data analysis methods.

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**Table 1: Performance of the algorithms on the applied data with shape of [**Dataset Shape: (555719, 23)**]**

| ALGORITHM | ACCURACY | PRECISION | RECALL | F1-SCORE |
|---|---|---|---|---|
| **XGBOOST** | **0.96** | **0.90** | **0.77** | **0.83** |
| **RANDOM FOREST** | **0.92** | **0.86** | **0.55** | **0.67** |

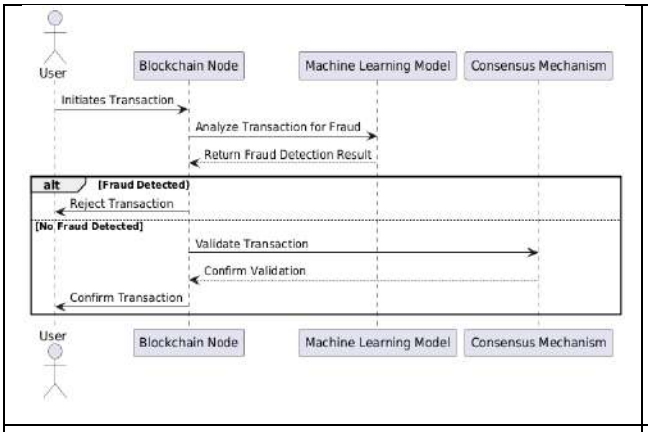


**Fig 1 : The Connection Between the User and the Application**

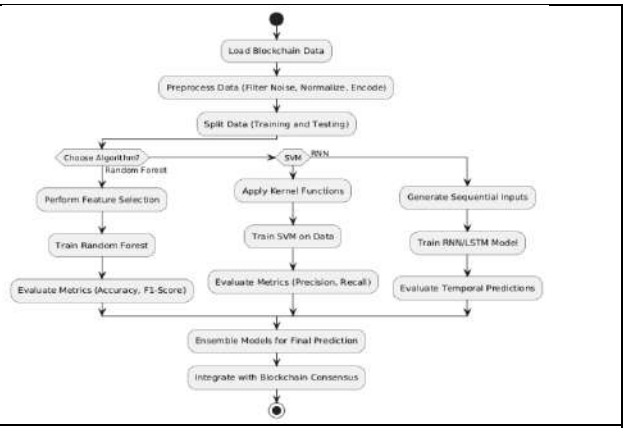


**Fig 2: Blockchain Technology Security Mechanism**

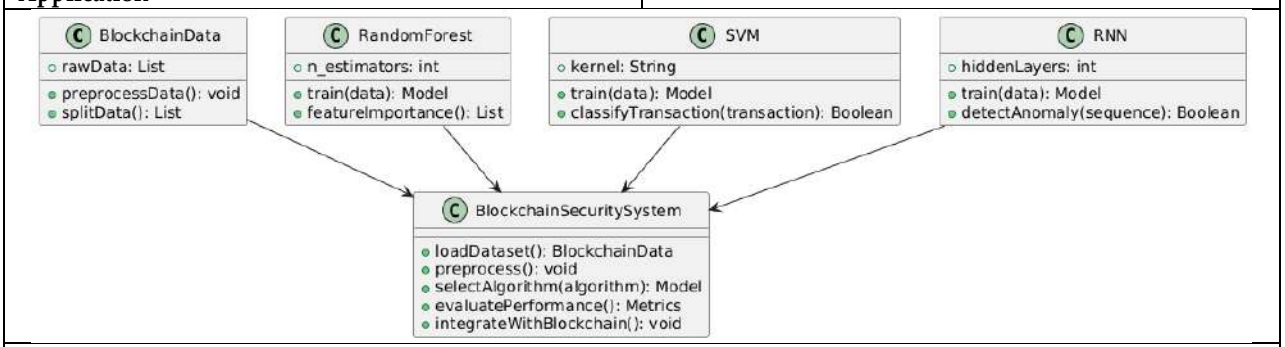


**Fig 3: Machine Learning(ML) Approach for BlockChain Security**

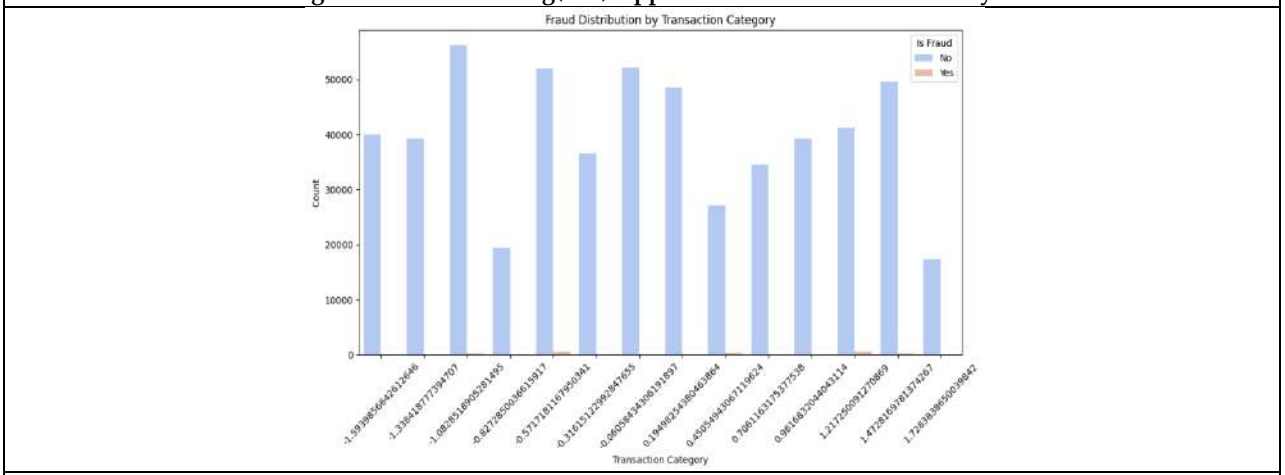


**Fig 4. : The Above Graph Shows the Fraud Detection Using the Applied Algorithms**

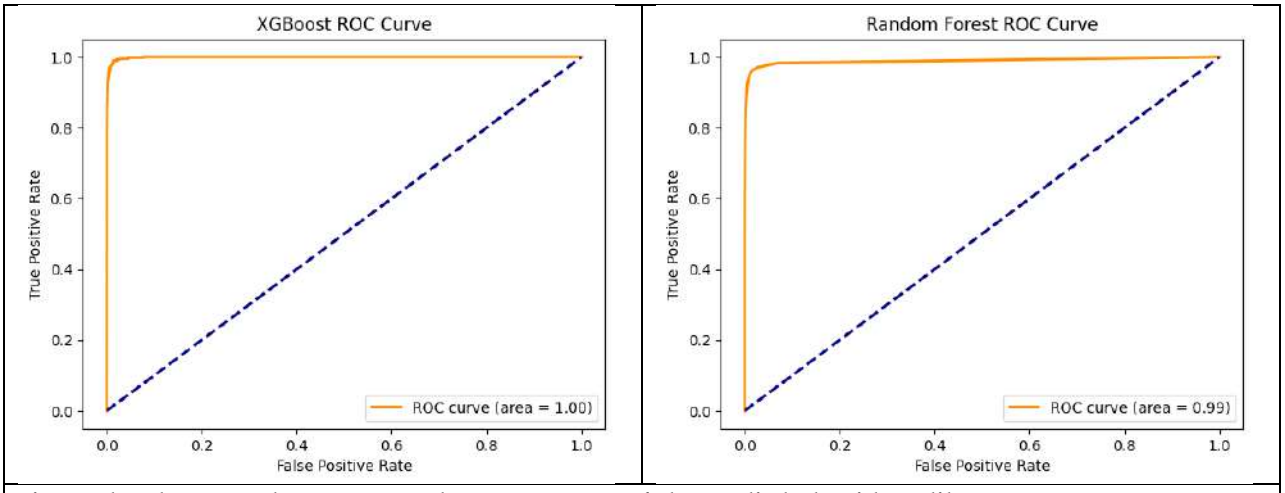


**Fig 5: The above graphs represents the ROC-Curves of the applied algorithms like XGBOOST & RANDOM FOREST**

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**RESEARCH ARTICLE**

# Assessing the Impact of Symptom Severity on Quality of Life in Thyroid Patients

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## ABSTRACT

The purpose of this cross-sectional descriptive study was to determine the extent to which the severity of symptoms affected the quality of life measures in people with thyroid disease. Studies carried out were undertaken at the Visakha Institute of Medical Sciences, and collected data from 155 thyroid patients, using the ThyPRO 39 questionnaire to assess quality of life. The findings showed that quality of life decreased with increasing age which could be due to age-related co morbidities. Other co morbidities like hypertension and diabetes mellitus added to this deterioration of the quality of life. Some other relevant symptoms that were highly reported were goiter, hyperthyroidism, hypothyroidism, eye problems, fatigue, memory loss, trouble, anxiety and depression, disturbance of emotions, trouble performing day-to-day activities, and cosmetic issues ($p < 0.05$). The above study raises numerous questions regarding the severity of symptoms versus the quality of life, and stresses the fact that in general, physical, psychological and social, factors must be controlled for in management. Still need further research on what were patient's coping strategies, what are their unfulfilled needs and what treatments they are ready for, in order to enhance patient's welfare.

**Keywords:** Symptom severity, ThyPRO questionnaire, Thyroid disease, Quality of life, Cross-sectional study, Endocrinology.

# INTRODUCTION

The thyroid gland is one of the types of endocrine glands which can be found in the inferior anterior neck region. It is involved in the formation as well as secretion of iodine homeostasis and thyroid hormones in the human body. It is estimated that the thyroid makes about 90 of inactive thyroid hormones or T4 while only about 10 is composed of active thyroid hormones which are referred to as T3 . Peripherally, inactive thyroid hormones are converted to the active form of thyroid which is also another inactive form of thyroid hormone. The normal balance and functions of the body systems are greatly influenced by the thyroid gland which enables normal growth and energy metabolism among other functions[1]. Thyroid dysfunctions are among the most common endocrine disorders especially in women. This disorder includes a wide range of problems including an underachieve the thyroid which is known as hypothyroidism and an above normal thyroid which is known as hyperthyroidism. These problems present themselves in a number of clinical features or symptoms which can be found in a range of systems in the body [2].

**Classification**

Subclinical hypothyroidism is a specific condition characterized by a slight increase in serum concentrations of thyroid-stimulating hormone (pituitary TSH), but with thyroid hormones remaining within the normal range. This diagnosis represents an initial of hypothyroidism which is not severe enough for a clinical diagnosis to be made and where the shortage of circulating thyroid hormones is not severe enough. It has been noted that up to 8 percent of patients with chronic subclinical hypothyroidism may go on to develop the fully blown hypothyroidism disease[3]. Subclinical hyperthyroidism is defined as low-serum TSH concentration along with normal serum concentrations of T4 and T3, in the absence of hypothalamic or pituitary disease, no thyroidal illness or consumption of drugs that suppress TSH secretion like glucocorticoids or dopamine[4]. Hypothyroidism, on the other hand, is a condition where there is insufficient thyroid hormone to regulate normal body functions. Over hypothyroidisms defined shaving both clinical symptoms and abnormal biochemical findings, with a prevalence varying from 118 individuals per thousand. Population studies show the ratio female/male to be between 2:1 and 8:1[5]. Hypothyroidism presents with symptoms including fatigue, weight changes, skin issues, and off voice[6]. A goiter is characterized by an enlarged thyroid gland ,exceeding the typical size limits suitable for a person based on age and gender (18mL for women, 25 mL for men).It is not a specific disease but a physical presentation with many different causes and physiologists. Furthermore, it can be associated with different metabolic states such as euthyroidism, hyperthyroidism, or hypothyroidism[7].Thyroid hormone is essential to our survival.Euthyroid Refers to an individual whose thyroid gland is working normally, as opposed to hyperthyroid (over active thyroid) or hypothyroid (under active thyroid[8].

**Thyroid quality of life (QOL) questionnaire**

A complete assessment tool is needed to understand QoL in hypothyroid patients. QoL is subjective and deeply rooted in effect, cognition, and behavior[9]. There is a consensus that quality of life (QoL) is negatively influenced by thyroid dysfunction, both in hyperthyroidism and hypothyroidism. One of the main aims in the therapy of thyroid dysfunction should be at least preserving or, ideally, improving QoL[10][11][12].For this reason, the measurement of health-related QoL has become an important issue of interest, and many instruments have been developed to measure this outcome parameter. QoL is often defined as a multidimensional subjective construct containing the dimensions of general health, physical, psychological, and social functioning[13]. Typical domains in QoL questionnaires include anxiety, impaired social life, or overall quality of life. Those more general domains are usually complemented in thyroid disease-specific questionnaires. The SF-36 questionnaire has become an important instrument for the assessment of health-related quality of life in different populations, including patients with thyroid disease [14]. The SF-36 has been significant in assessing a range of health dimensions, including physical functioning to mental health, in studies of thyroid patients[15].Clearly, though, the correct authors can say that summary

measures such as the Physical Component Summary (PCS) and Mental Component Summary (MCS) can only be computed using proprietary algorithms maintained by resource providers. These graphs highlight the importance of making sure they have been properly licensed and their usage protocols[16].

**ThyPRO Questionnaire**

The Thyroid-Related Patient-Reported Outcome (ThyPRO) questionnaire is proposed, evaluating the quality of life in patients with non-malignant thyroid disease. This well validated instrument encompasses a variety of benign thyroid ailments: hypothyroidism, hyperthyroidism, goiter and orbitopathy.ThyPRO is a powerful tool to evaluate the effect of thyroid disorders on patients 'well-being and provides a broad perspective on thyroid-related symptoms and experiences[17][18][19][20] . Managing thyroid disease focuses on reducing symptom impacts and improving HRQOL. Patient-reported outcomes, like HRQOL and symptom severity, are key metrics[21][22]. ThyPRO is an 85-item questionnaire assessing thyroid symptoms and HRQOL. It is the most used tool for thyroid dysfunction, with strong reliability and validity.

ThyPRO-39, a shorter version with 39 items, retains strong psychometric properties. It is effective and user-friendly in clinical settings [23][17][18]. A recent study titled "Assessing the Impact of Symptom Severity and Burden on Quality of Life in Thyroid Patients" delves into thyroid disorders such as hypothyroidism and hyperthyroidism. It underscores the necessity of longitudinal evaluations to follow how symptoms evolve over time and underscores the role of patient-reported outcomes (PROs). They provide case studies of the treatment and age/gender differences and psychosocial factors to enhance thyroid patient care.

**OBJECTIVES**

1. Identify moderating factors, such as age, comorbidities, hormone levels, disease duration, and weight, that may impact the link between symptom severity and quality of life.
2. Determine the severity, frequency, and duration of various symptoms in thyroid disease patients.
3. Assessing the total quality of life in thyroid disease patients, including physical, emotional, and social well-being.
4. Examine the relationship between symptom severity and quality of life in thyroid patients.

## MATERIALS AND METHODS

**Study Design**: The study is a hospital based Cross-Sectional Analytical Study at a tertiary care teaching hospital.
**Study Duration**: 6 months (October 2023- March 2024).
**Study Subjects**: The study is carried out by considering the following criteria:
**Inclusion criteria**:

- Thyroid Patients
- Adults (aged 18-75 years)
- Consent and Participation
- Ability to Respond

**Exclusion criteria**:

- Severe Illness
- Cognitive Impairment
- Pregnancy/Breastfeeding
- Unwillingness to Participate

**Sampling technique:** Stratified Random Sampling technique was applied to select the study subjects.

**Sample size:** Sample size is calculated by using Taro yamen sample size calculator with a 0.05% margin of error and 95% confidence level. The recommended sample size is 155 for a population size of 260 members.

**Ethical considerations:**
Both the literate and illiterate patients were clear about the aim and objectives of the research work and informed consent was obtained from them upon their willingness to participate in the study. No experiments are being conducted on the patient; therefore, there are no risks or compensation.

**Study instruments:**
**ThyPRO 39 scale**
We have used this scale to interpret the overall quality of life in thyroid patients using the overall scoring. To categorize the overall score of the ThyPRO questionnaire, we had combined scores of all the domains and provided an interpretation. The questionnaire consists of 12 domains. Each domain was scored on a scale of 0(Not at all), 1(somewhat), 2(very much) using Likert scale. The interpretation of overall scoring of ThyPRO questionnaire was 0-25(Low impact), 26-50(Moderate impact), 51-75(High impact), greater than 76(Very high impact).

**Data collection:**
Data was collected from handwritten prescription by the physician and patient interview which includes questions related to patient demographics, co morbidities; symptoms of goiter, hyperthyroid, hypothyroid, eye, and; tiredness, cognitive, anxiety, depressively, emotional susceptibility, impaired social life, impaired daily life, cosmetic complaints, using ThyPRO 39 questionnaire

**Data analysis:**
Frequencies and percentages were calculated for qualitative data. Mean and standard deviation was calculated for quantitative data. Chi square test was employed to find any statistically significant associations between symptoms and quality of life in thyroid patients. Logistic regression was also employed to find statistically significant association between age, weight, hormone levels (T3, T4, and TSH), co morbidities, duration of disease and quality of life in thyroid patients. Jeffrey's Amazing Statistical Program (JASP version 0.18.0) software was used for statistical analysis. A p-value <0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

This study aims to delve deeper into the complexities of thyroid disorders, particularly focusing on the multitude of factors that may influence the relationship between symptoms and quality of life. By exploring variables such as age, co morbidities, hormone levels, duration of disease, and weight, the research seeks to shed light on the underlying mechanisms that contribute to patients' ongoing symptomatic experience and its impact on their overall well-being. With this goal in mind, the current study was undertaken at Visakha Institute of medical sciences, catering to a sample of approximately 155 patient sample size employed in this investigation, using thyPRO39 questionnaire, data was collected from the patients and interpretated accordingly. One of the primary objectives of the study is to investigate potential moderating factors that may influence the relationship between symptom severity and quality of life in thyroid patients. By examining factors such as age, co morbidities, hormone levels, duration of disease, and weight, the study seeks to identify variables that may exacerbate or alleviate the impact of symptom severity on quality of life. Additionally, the study aims to assess the severity of individual symptoms experienced by patients with thyroid disease. This involves examining the intensity, frequency, and duration of symptoms such as tiredness, cognitive issues, anxiety, depressively, and impaired daily life. By quantifying the severity of these symptoms, the study provides valuable insights into the overall symptom burden experienced by thyroid patients.

The observed decrease in quality of life among older thyroid patients (p-value <0.01) could be attributed to various age-related factors that were not adequately explored in the literature. As individuals age, they may experience a higher burden of co morbidities, such as cardiovascular disease, arthritis, or cognitive decline, which can significantly impact their quality of life. Additionally, aging is often associated with changes in hormonal levels. decreased physical function, and increased susceptibility to psychological distress, all of which can contribute to a decline in overall well-being. Furthermore, older individuals may face social and environmental challenges, such as limited mobility, reduced social support networks, and financial constraints, which can further exacerbate the impact of thyroid disease on their quality of life. While the duration of thyroid disease may also influence quality of life, the lack of literature evidence and insufficient data in our study precluded a comprehensive assessment of this factor. However, it is plausible that individuals with longer-standing thyroid conditions may experience greater disease-related burden and symptom severity, potentially leading to a more pronounced impact on their quality of life over time. Further research exploring the interplay between age, disease duration, and quality of life in thyroid patients is warranted to better understand these complex relationships and inform targeted interventions aimed at improving outcomes in this population.

In our study, patients with hypertension and diabetes mellitus as co morbidities showed higher chances of deprivation of quality of life with p-value<0.01, compared to those without co morbidities. The presence of multiple co morbidities, such as HTN and DM2, often exacerbates the burden on individuals with treated hypothyroidism, leading to a further reduction in HRQoL. This additive effect suggests that the concurrent management of co morbid conditions alongside thyroid disorders is crucial for optimizing patients' well-being. Scientifically, this could be explained by the intricate interplay between physiological mechanisms underlying both thyroid dysfunction and co morbidities such as HTN and DM2. Similarly, the highly significant association between goitre symptoms and quality of life in thyroid patients (p-value<0.01) underscores the potential impact of goitre on patients' overall well-being. However, the scarcity of literature on this relationship indicates a need for further investigation into the specific effects of goitre symptoms on quality of life. Future research should explore the underlying mechanisms driving this association and evaluate interventions aimed at alleviating goitre symptoms and improving patients' overall quality of life.
In our findings, the highly significant association (p <0.01) observed between hyperthyroid symptoms and quality of life underscores the profound impact of thyroid dysfunction on mental health. This is supported by research showing a higher prevalence of psychiatric disorders in individuals with hyperthyroidism. The improvement noted after endocrinological treatment suggests a direct link between thyroid hormone levels and mental well-being. Routine screening for mental health symptoms in hyperthyroid patients is crucial for timely intervention. Future longitudinal studies are needed to explore the temporal correlation between psychiatric symptoms and hyperthyroidism, and impact on overall quality of life.

In our findings it is revealed that a significant association exist between hypothyroid symptoms and quality of life with p value of 0.01. These findings are consistent with the prior studies stating that there is a positive correlation between hypothyroid symptoms and quality of life, as indicated by significant composite scores of ThyPro39 (p<0.01). Another prior investigation indicates a non-significant direct relationship between quality of life and hypothyroid symptoms, suggesting that while both factors independently influence ThyPro39 scores, their interaction may be influenced by moderating factors or the intricate nature of their relationship. One plausible explanation for this discrepancy could be the presence of moderating factors or the intricate nature of the relationship between symptoms and quality of life. For instance, other variables such as age, co morbidities, or hormone levels may influence how individuals perceive and report their symptoms, consequently impacting their quality-of-life scores

In our study, the significant association (p <0.01) between eye symptoms and their impact on quality of life in thyroid patients highlights the profound influence of ocular complaints on overall well-being. This finding aligns with previous research indicating impairments in quality of life among patients with benign thyroid

disorders, particularly regarding visual complaints. The positive relationship observed between scores of the "ocular complaints" scale and other symptoms such as depression, anxiety, cognitive disorders, and general quality of life underscores the interconnectedness of subjective visual disturbances with various aspects of patient well-being. Scientifically, this association may be attributed to the intricate neural connections between the visual system and areas of the brain involved in emotional regulation, cognition, and overall perception of health. Addressing ocular complaints through specialized eye care interventions could thus potentially alleviate the burden of thyroid-related symptoms and improve overall quality of life for affected patients.

The impact of tiredness on the quality of life in thyroid patients remains inconclusive due to a lack of available literature directly linking the two. While fatigue is commonly reported by thyroid patients, the exact relationship between tiredness and quality of life is poorly understood. Factors such as thyroid hormone imbalances, autoimmune processes, and psychological factors like depression and anxiety may contribute to fatigue in thyroid patients. However, further research is needed to clarify the precise mechanisms underlying this relationship and to quantify the impact of tiredness on quality of life in thyroid patients. With our findings, highly significant association ($p < 0.01$) is observed between emotional susceptibility and its impact on thyroid patient overall quality of life which underscores the profound influence of emotional factors on individuals with thyroid disorders. Scientifically, thyroid hormones regulate neurotransmitter levels in the brain, affecting mood regulation, and deregulation of these hormones can lead to mood disturbances such as anxiety and depression. Additionally, thyroid dysfunction can impact the hypothalamic-pituitary-adrenal axis, exacerbating emotional susceptibility and impairing coping mechanisms. This emphasizes the importance of addressing emotional well-being alongside traditional treatments for thyroid disorders to optimize patient outcomes.

In our findings, a significant association ($p<0.01$) between impaired daily life and overall quality of life in thyroid patients highlights the profound influence of impaired functioning on overall well-being. Thyroid diseases like hypo and hyperthyroidism, significantly affect patients' ability to work due to symptoms like fatigue, cognitive impairment, and mood disturbances. This leads to difficulties in managing professional responsibilities, with many unable to return to previous employment even after remission.(Ragusa et al., 2019)Female patients, in particular, face challenges in returning to work promptly and often reduced workloads compared to males. In summary, thyroid diseases impair daily functioning and work ability due to symptom severity and gender related factors, thereby impacting overall quality of life. Comprehensive support and interventions are essential to address these impairments and improve patient outcomes. In our findings, Cosmetic complaints have a significant association (0.001) with the impact on overall quality of life. The symptoms that have the strongest positive correlation with the impact on overall quality of life are impaired daily life, and emotional susceptibility. Impaired social life does not seem to have a direct relationship with the overall quality of life. We can observe that certain symptoms have a stronger correlation with the impact on overall quality of life. For instance, "Impaired daily life" shows notable correlations. This suggests that these symptoms significantly affect the overall quality of life of individuals experiencing them. Due to limited studies being conducted on assessing the impaired daily life, cosmetic complaints, faced by thyroid patients, we are unable to provide evidence which may prove that these may show a significant impact the overall quality of life in thyroid patients.

In our study we assessed the impact of symptom severity on quality of life in thyroid patients and revealed significant association regarding age i.e. increase in age thereby decreases quality of life in thyroid patients. Patients with both hypertension and diabetes mellitus as co morbidities add up to deterioration of quality of life in thyroid patients. Symptoms including Goiter, Hyperthyroid, Hypothyroid, Eye symptoms, Tiredness, Cognitive symptoms, Anxiety,Depressivity, Emotional Susceptibility, Impaired Social Life, Impaired Daily Life, Cosmetic Complaints,Impact on Overall Quality of Life marked significant impact to quality of life in thyroid with p value $<0.05$. The study proposes importance of addressing further qualitative research to

explore patients 'perspectives and experiences regarding the impact of thyroid dysfunction on their quality of life, including coping mechanisms, treatment preferences, and unmet needs. All the relevant data collected and interpreted information was given in Tables 1,2 & 3.

**Limitations**

While our study design appears comprehensive, several limitations can be acknowledged. Firstly, the use of a cross-sectional design limits the ability to establish causal relationships between variables. Additionally, reliance on self-reported data from patients may introduce response bias or recall bias, impacting the accuracy of reported symptoms and quality of life assessments. The study's duration of six months may not capture seasonal variations or long-term changes in patient symptoms. Furthermore, the use of a single instrument, the ThyPRO 39 scale, for data collection may overlook nuanced aspects of thyroid-related symptoms and quality of life. Moreover, the exclusion of patients with severe illness or cognitive impairment may lead to a biased sample, potentially overlooking important perspectives from these populations. Finally, the study's setting in a single tertiary care teaching hospital may limit the generalizability of findings to broader populations with thyroid disorders.

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**Table 1: Quantitative variables for different patient populations**

| S.no | Characteristic | Mean±SD |
|---|---|---|
| 1 | Age | 42.75±12.61 |
| 2 | Weight | 63.70±13.00 |
| 3 | Duration of disease | 5.93±5.44 |
| 4 | T3 | 5.56±20.88 |
| 5 | T4 | 8.08± 2.63 |

| | | |
|---|---|---|
| 6 | TSH | 12.70± 23.92 |
| 7 | Goitre Symptoms | 1.50 ±2.10 |
| 8 | Hyperthyroid Symptoms | 2.74 ±2.14 |
| 9 | Hypothyroid Symptoms | 1.92±1.97 |
| 10 | Eye Symptoms | 1.72±1.90 |
| 11 | Tiredness | 3.11±1.45 |
| 12 | Cognitive | 1.43±2.01 |
| 13 | Anxiety | 2.23±2.16 |
| 14 | Depressivity | 2.19±1.63 |
| 15 | Emotional Susceptibility | 2.21± 1.55 |
| 16 | Impaired Social Life | 0.19±0.55 |
| 17 | Impaired Daily Life | 1.83±1.99 |
| 18 | Cosmetic Complaints | 1.49±1.18 |
| 19 | Overall Scoring | 23.78±11.32 |

**Table 2 : Association between different variables and overall interpretation of quality of life**

| **Characteristic** | **Overall Interpretation** | | | **P-value** |
|---|---|---|---|---|
| | **High Impact** | **Low Impact** | **Moderate Impact** | |
| **Gender** | | | | 0.27 |
| Female | 10 | 62 | 72 | |
| Male | 0 | 8 | 3 | |
| **Menstruation** | | | | 0.30 |
| N/A(Males) | 0 | 8 | 3 | |
| Irregular | 3 | 8 | 12 | |
| Regular | 3 | 30 | 27 | |
| Menopause | 4 | 24 | 33 | |
| **Co morbidities** | | | | <0.01 |
| Asthma, HTN | 1 | 0 | 0 | |
| Asthma, DM2 | 0 | 0 | 1 | |
| DM2 | 2 | 13 | 6 | |
| HTN | 0 | 2 | 11 | |
| HTN, DM2 | 1 | 12 | 12 | |
| Nil | 6 | 40 | 43 | |
| Hyperlipidemia | 0 | 1 | 0 | |
| Hypotension | 0 | 0 | 1 | |
| Migraine | 0 | 0 | 1 | |
| PCOD | 0 | 1 | 0 | |
| Rheumatoid Arthritis | 1 | 0 | 0 | |
| **Disease type** | | | | <0.01 |
| D Euthyroid | 0 | 0 | 1 | |
| Euthyroid | 0 | 1 | 0 | |
| Goitre | 0 | 0 | 1 | |
| Graves | 0 | 0 | 1 | |
| Hyperthyroidism | 0 | 2 | 4 | |
| Hypothyroidism | 2 | 8 | 10 | |

| | | | | |
|---|---|---|---|---|
| Primary Hypothyroidism | 8 | 57 | 56 | |
| Secondary Hypothyroidism | 0 | 2 | 2 | |
| **Goitre Category** | | | | <0.01 |
| Low | 0 | 0 | 1 | |
| Mild | 4 | 67 | 49 | |
| Moderate | 1 | 1 | 11 | |
| Severe | 5 | 2 | 14 | |
| **Hyperthyroid Category** | | | | <0.01 |
| Mild | 0 | 56 | 24 | |
| Moderate | 7 | 13 | 34 | |
| Severe | 3 | 1 | 17 | |
| **Hypothyroid Category** | | | | 0.01 |
| Mild | 4 | 58 | 46 | |
| Moderate | 4 | 9 | 22 | |
| Severe | 2 | 3 | 7 | |
| **Eye Category** | | | | <0.01 |
| Mild | 4 | 63 | 48 | |
| Moderate | 2 | 5 | 20 | |
| Severe | 4 | 2 | 7 | |
| **Tiredness Category** | | | | <0.01 |
| High | 1 | 1 | 8 | |
| Low | 0 | 40 | 20 | |
| Moderate | 9 | 29 | 47 | |
| **Cognitive Category** | | | | <0.01 |
| Mild | 1 | 68 | 50 | |
| Moderate | 4 | 1 | 14 | |
| Severe | 5 | 1 | 11 | |
| **Anxiety Category** | | | | <0.01 |
| High | 7 | 1 | 19 | |
| Low | 0 | 61 | 30 | |
| Moderate | 3 | 8 | 26 | |
| **Depressivity Category** | | | | <0.01 |
| High | 5 | 1 | 7 | |
| Low | 2 | 63 | 43 | |
| Moderate | 3 | 6 | 25 | |
| **Emotional Susceptibility Category** | | | | <0.01 |
| High | 5 | 0 | 5 | |
| Low | 1 | 64 | 47 | |
| Moderate | 4 | 6 | 23 | |
| **Impaired Daily Life Category** | | | | <0.01 |
| High | 5 | 2 | 8 | |
| Low | 3 | 61 | 42 | |
| Moderate | 2 | 7 | 25 | |
| **Cosmetic Complaints Category** | | | | <0.01 |
| High | 1 | 0 | 1 | |

| | | | | |
|---|---|---|---|---|
| Low | 7 | 70 | 69 | |
| Moderate | 2 | 0 | 5 | |

**Table 3: Logistic regression analysis of various variables and quality of life**

| Variable | Odds Ratio | 95% CI | P-value |
|---|---|---|---|
| Age | 1.024 | 1.001 - 1.047 | 0.041 |
| Duration of disease | 0.986 | 0.952 - 1.021 | 0.431 |
| Hypertension | 1.279 | 0.675 - 2.421 | 0.449 |
| Diabetes mellitus type 2 | 1.871 | 0.994 - 3.521 | 0.052 |
| Hypertension and Diabetes mellitus type 2 | 2.164 | 1.074 - 4.358 | 0.031 |
| Weight | 1.011 | 0.997 - 1.026 | 0.136 |
| T3 level | 0.848 | 0.684 - 1.051 | 0.132 |
| T4 level | 1.071 | 0.961 - 1.194 | 0.214 |
| TSH level | 1.004 | 0.979 - 1.030 | 0.740 |

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**RESEARCH ARTICLE**

# Preliminary Phytochemical Screening and *Invitro* Pharmacological Activities of *Limonia Acidissima* Fruits

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## ABSTRACT

Wood apple fruit, or *Limonia acidissima*, is edible and utilized in a very small number of culinary recipes. This study investigates the phytochemical, antioxidant, antidiabetic, and antimitotic properties of *Limonia acidissima* fruit extract. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, and amino acids. The antioxidant potential was evaluated using the DPPH radical scavenging assay, which demonstrated dose dependent activity. The extract exhibited a maximum inhibition of 40.17% at 10 mg/mL, with an IC50 value of 2.8619 mg/mL, outperforming the standard ascorbic acid (IC50: 3.6619 mg/mL). The antidiabetic activity was assessed using a glucose uptake assay in a yeast cell model. At 10 mg/mL, the extract facilitated 98.84% glucose uptake, significantly higher than the standard drug metronidazole (70.5%). This activity is attributed to the stereospecific membrane carriers that enhance glucose transport, suggesting the extract's potential in regulating blood glucose levels. The antimitotic activity of *L. acidissima* was studied using seed germination assays, demonstratinga dose-dependent suppression of seed development. The methanolic extract at 40 mg/mL exhibited an inhibition similar to that of the common medication methotrexate, with percentage inhibition of the seed germination recorded as significant across various time points (24, 48, and 72 hours). Collectively, the findings indicate that *L. acidissima* exhibits strong antioxidant, antidiabetic, and antimitotic properties, suggesting its potential as a source for developing therapeutic agents targeting oxidative stress, diabetes, and cancer. Further studies are recommended to explore its bioactive constituents and underlying mechanisms.

**Keywords**: Antioxidant, Antidiabetic, Anti mitotic, Wood apple, Fruit .

# INTRODUCTION

The wood apple, or *Limonia acidissima*, is a deciduous, upright tree that can reach a height of 15 meters and has drooping branches. The flowers are grouped in terminal or lateral panicles and are a dull red color. Fruits are round to oval, 5-15 cm in diameter, with a firm, woody, grayish-white, scurfy skin and whitish pulp (when unripe); they turn brown when mature, and the pulp contains many tiny, white seeds that are odorous, acidic, or sweet. The huge tree *Limonia acidissima* L. reaches up to 9 meters (30 feet) in height and has rough, prickly bark. The axillary, thin sharp spines on some of the zigzag twigs are 2 to 5 cm long. With five to seven leaflets, each measuring 25 to 35 mm in length and 10 to 20 mm in width, the pinnate leaves have a citrusy aroma when crushed. A terminal leaflet and two to three opposing leaflets in pairs. A berry with a diameter of 5 to 9 cm, the fruit can be either sour or sweet. Its extremely tough rind might be challenging to break through. It has a sticky, dark, and fragrant pulp. It has a lot of tiny, white seeds strewn throughout and is repulsive, astringent, acidic, or sweet. The rind is 6 mm thick and has a greyish-white hue. Its exterior shell, known as the rind, is woody and incredibly hard, making it quite difficult to break apart. The hard skin of wood-apple fruit, which is 5–6 mm long, hairy, and has thick, green cotyledons, is cracked with a hammer; germination occurs epigeally. *Limonia acidissima* L. is a common tree. This is a tree of intermediate size, reaching a height of 9 meters (30 feet). Usually, it is a deciduous kind.

**Phytochemicals**

Tri-terpenoids, amino acids, tannins, coumarins, polyphenols, vitamins, and saponins are among the phytochemicals found in wood apples [1] (Table 1). Ripe fruits of the Limonia acidissima plant include flavonoids, steroids, glycosides, and other acidic chemicals, according to phytochemical study. Acidissimin and acidissiminol are the two main chemical components of leaves. It is also observed that leaves contain gum and mucilage, phenols, resins, alkaloids, and fixed oils and fats [2-5]. The pulp from wood apples is a very good source of dietary fiber (1.7%), protein (13.8%), fat (4.3%), and carbs (70.14%) [1,4]. This fruit is also believed to have significant levels of zinc, calcium, magnesium, iron, and a low fat content (4.38%). High levels of calcium and phosphorus are also discovered, and these are crucial for blood coagulation, bone formation, and other metabolic functions. Iron in fruit is a sign of its ability to combat anemia, TB, and other illnesses [6]. Screening for antioxidant activity has been done on various sections of *Limonia acidissima* L.Crude methanolic extract has the strongest free radical scavenging activity measured by antioxidant activities of plant components on the stable radical DPPH. Additionally significant antioxidant activity was demonstrated by the pet ether soluble fraction (PE). *Limonia* fruit methanolic extract was also tested using the FRAP and DPPH assays to see whether it had the ability to scavenge free radicals. Various leaf extracts from Limonia acidissima L. have been documented.

**Pharmacological studies**

**Antihyperlipidemic activity**

Hepatic glucose-6-phosphatase and lipid profile are decreased after 28 days of administering fruit powder at 2.5, 5, and 10 g/Kg body weight, but hepatic glycogen, hexokinase, and HDL are significantly increased.This could be caused by the presence of fibers, ascorbic acid, flavonoids, polyphenols, saponins, and phytosterols [7].

**Hepatoprotective activities**

Rats' liver injury from carbon tetrachloride (CCl4) was tested to determine the hepatoprotective potential of methanolic pulp extracts. Following three days of intraperitoneal administration of 100, 200, and 400 mg/kg to rats, they found that hepatic enzyme levels, specifically those of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AST), total protein (TP), total bilirubin (TB), and gamma-glutamyltransferase (GGT), increased while those of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) declined. A different study found that aqueous leaf extract increases the serum concentrations of alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). In comparison to the normal control, it also raises the levels of urea, creatinine, potassium, and sodium in the rats that are not treated for phenyl

hydrazine-induced anemia [8].

**Anticancer Activity**

*Limoniaacidissima* L. has been demonstrated, it's fruit extract has anticancer effects. In two distinct breast cancer cell lines, SKBR3 and MDAMB-435, the ED50 value (50 percent inhibition of cancer cell growth) was determined using ethanolic fruit extracts [9].

**Antibacterial Activity**

According to reports, ethanolic extract from *Limonia acidissima* L. leaves exhibits extremely extensive spectrum of action against both Gram-positive and Gram-negativebacteria, which are the main cause of bacterial illnesses.

**Neuroprotective activities**

Neuroprotective effects of wood apple was evaluated and showed that at 250 mg/kg and 500mg/kg body weight it inhibits ischemia reperfusion-induced brain injury in rats [10].

**Analgesic activity**

Using methanol and fruit peel acetone extracts, the analgesic activity was 60.53% and 59.65%, respectively, compared to 78.07% using the conventional medication Diclofenac Na to treat writhing mice induced by acetic acid [11].

**Diuretic activity**

The leaves methanolic extract has considerably increased urine production.The extraction process establishes the quantity of urine and electrolyte excretion, particularly that of sodium, potassium, and chloride ions. In comparison to Bath Sonicator extraction (BSE), it was discovered that Microwave assisted extraction (MAE) exhibited superior activity [12].

## MATERIALS AND METHODS

**Materials:** DPPH, Ethanol, Ascorbic acid,Baker's yeast, Glucose, Methanol, Metronidazole, Vincristine sulfate Injection.All the chemicals used here are of analytical grade.

**Collection of plant material:** The fully mature fruits of *Limonia accidissima* was collected in the months of July to august 2024 from Sambavaram, Visakhapatnam, India. The fruits were made into small pieces, oven dried at $40^0$C for 7 days and converted into coarse powder with an auto mix blender.

**Preparation of Methanolic extract**

1 kg powder of each sample was taken in a round-bottomed flask and the methanolwas added to the powdered drug and it was kept for reflux extraction for 3hrs. The extracts wasconcentrated under a vacuum to get the corresponding residue and the tests were performed.

**Preliminary Phytochemical Analysis**: To verify the existence of phytochemicals like alkaloids, flavonoids, terpenoids, amino acids, cardiac and anthracene glycosides, saponins, steroids, tannins, phenols, and carbohydrates, a preliminary phytochemical study was conducted utilizing qualitative testing.

***In vitro* Antioxidant activity DPPH Radical Scavenging Activity (DPPH)**

Using the stable DPPH method [13], the extracts' ability to scavenge free radical DPPH was assessed. Five different concentrations (ranging from 25 to 200 μg/mL) of each extract were made. To 1 mL of sample solution with varying concentrations, 4 mL of DPPH at 0.1 mM solution was added. The ELICO SL210 UV double-beam spectrophotometer was used to measure the absorbance at 517 nm following a 30-minute incubation period. The standard and controlhad the same levels of the synthetic antioxidant ascorbic acid (25–200 μg/mL), and the blank solution was devoid of extract or standard. The DPPH scavenging activity increases as the samples' absorbance decreases. Each extract's IC50 value was determined by plotting a standard curve.

% inhibition of DPPH radical = $\frac{AbsControl\ -AbsSample}{AbsContol} \times 100$

***In vitro* Antidiabetic activity by glucose uptake method:**

To measure the uptake of glucose in yeast cells, the unique method was used. Commercially available *Saccharomyces cerevisiae* (baker's yeast) cells were suspended in 1% solution and kept at 25°C [14]. The supernatant fluid was produced by continuously centrifuging overnight yeast cell solution at 4,200 rpm for 5 minutes. The clear supernatant was used to prepare a 10% v/v yeast cell culture for the experiment. Standard drug (MTZ) and plant samples at different concentrations (100–500 µg/mL) were incubated for 10 minutes at 37°C using 1 mL of a 10 mM glucose solution. The reaction was initiated by adding 100 µL of yeast suspension, vortexing the liquid, and then incubating it for 60 minutes at 37°C.

Following that, the samples were centrifuged for five minutes at 3,800 rpm. By using a UV-vis spectrophotometer set to 520 nm [15], the amount of glucose in the supernatant was determined. Every reagent except the test sample is present in the control sample. The full experiment was conducted three times. The proportion of glucose absorption was calculated using the formula.

% of glucose uptake = $\frac{AbsControl\ -AbsSample}{AbsControl} \times 100$

***In vitro* Antimitotic activity by plant-based models**

We collected and weighed green gram seeds separately from the local supermarket. Extract concentrations were generated at 10 mg/mL, 20 mg/mL, 30 mg/mL, and 40 mg/mL. The standard used here is methotrexate. We utilized distilled water as a control. To the material in Petri plates with varying concentrations, equal weights of seeds were introduced. For the purpose of imbibitioning water, the Petri plates were kept at room temperature for a full day. On dry tissue paper, the drug treatment was dried and weighed after 24 and 72 hours. The sprouting period was prolonged to 72 hours, and pictures were captured.

% inhibition = $\frac{Weight\ of\ seeds\ in\ distill\ water\ -weight\ of\ seeds\ in\ extract\ sample}{Weight\ of\ seeds\ in\ distilled\ water\ -weights\ of\ seeds\ in\ methotrexate}$ X 100

## RESULTS AND DISCUSSIONS

**Preliminary Phytochemical Analysis**

Alkaloids, amino acids, coumarins, carbohydrates, tannins were among the phytochemicals present in *Limonia* fruit extract (Table 2)

***In vitro* Antioxidant activity DPPH Radical Scavenging Activity (DPPH)**

In a dose-dependent manner, the extract demonstrated inhibition at doses ranging from 2 to 10 mg/mL. The amount of antioxidants needed in the sample to 50% inhibit free radicals is known as the IC50 value. The IC50 value decreases as radical scavenging capacity increases. While the positive standard ascorbic acid demonstrated 52.14% inhibition and an IC50 value of 3.6619 mg/mL, the extract demonstrated action in a dose-dependent manner with a maximum inhibition of 40.7% at 10 mg/mL and an IC50 value of 2.8619 mg/mL. The graph above showed the percentage reduction in DPPH scavenge activity resulting from *Limonia acidissima* (Table 3 & Fig 1).

**Anti diabetic by glucose uptake method**

*L. acidissima* fruit extract with varying concentrations wasevaluated for in vitro antidiabetic efficacy utilizing a yeast model and the glucose uptake assay. The extract showed more action than the others (98.84% at 10 mg/mL) concentrations of extract. An increase in glucose percentage that is dose-dependent and uptake with increasing concentration (2-10 mg/mL). *L. acidissima* fruit extract was observed in the above graph. The standard drug Metronidazole showed 70.5% at 10 mg/mL

***In vitro* Antimitotic activity by plant based models**

The assay's outcomes demonstrated that, following 24 hours, 48 hours, and 72 hours of treatment, the methanolic extract of green grams drastically raised the percentage inhibition, which was comparable to that of methotrexate. The standard chemotherapy drug Methotrexate, at 0.1 mg/ml, inhibited seed germination by 0.001±1.0 percent, but the methanolic extract at 40 mg/ml showed seed growth of 0.01±0.2 mm. In comparison to the percentage of inhibition of methotrexate, the assay findings demonstrated that the methanolic extract of green grams considerably reduced the percentage of inhibition after 24 hours, 48 hours, and 72 hours of treatment. The methanolic extract of concentration 20mg/ml showed seed growth of 0.88±0.8mm. In the point of view of seed germination, the control i.e., distilled water has shown the higher seed germination compared to standard and other methanolic extracts (Table 4).

## CONCLUSION

*L. acidissima* fruit extract was extracted and subjected to preliminary phytochemical screening in this investigation. Alkaloids, carbohydrates, and amino acids are detected via screening. The antioxidant activity of the fruit extract from *L. acidissima* was one of the objectives of the current investigation.Since the DPPH method's results are unaffected by the polarity of the substrate, it was regarded as a stable technique. The primary mechanism by which the beneficial ingredients in plant extracts scavenge free radicals may be because they impart hydrogen to the free radicals, neutralizing them and limiting their deadly effects by generating stable molecules.The extract's IC50 was 2.8619 mg/mL, and its maximum DPPH scavenging activity was 40.17 mg/mL. In contrast, the standard IC50 was 3.6619 mg/mL.Using yeast cells to measure glucose absorption, an effective method to evaluate antidiabetic efficiency in vitro, *L. acidissima* was tested for antidiabetic activity.

One of the most obvious markers of glucose uptake by yeast cells was the amount of glucose that remained in the medium after a specific period of time. Using a yeast cell model, *L. acidissima* extract's in vitro antidiabetic action was demonstrated by showing that, at 10 mg/ml, the percentage of glucose uptake by yeast cells mediated by the plant extracts was 98.84%, while the percentage of metronidazole was 70.5%. The diffusion process, assisted by stereospecific membrane carriers, facilitated the glucose movement through the yeast cell membrane. This demonstrated clearly that *L. acidissima's* methanol extract absorbs glucose more efficiently by improving its utilization, which in turn helps regulate blood glucose levels. This investigation on *L. acidissima's* antimitotic activity indicates that the plant may have antimitotic properties. As a result, this plant may produce drugs that could be utilized to treat cancer. The percentage inhibition following 24 hours, 48 hours, and 72 hours of therapy was shown to be much higher with the methanolic extract of green grams than with methotrexate. With an antimitotic activity of 0.01±0.2 mm at 40 mg/ml methanolic extract, the percentage inhibition of seed germination was as strong as that of the common medication Methotrexate, which is 0.001 mg/ml±1.0 mm.

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**Table 1. Phytochemicals in Wood apple**

| S.No | Phytochemicals | Part |
|---|---|---|
| 1 | Flavonoids, Phenols | Fruits, Stem bark |
| 2 | Tannins, Flavonoids | Leaves |
| 3 | Essential Oil | Fruit Pulp |
| 4 | Polar Constituents | Bark |
| 5 | Acidic Polysaccharide | Fruit, Pulp |
| 6 | Tannins, Steroids, Flavanol, Alkaloids | Leaves |
| 7 | Essential Oil | Fruit, Stem bark, Fruit Pulp and Leaves |

**Table 2. Phytochemicals present in Wood apple fruit extract**

| S.No | Name Of the test | Observation |
|---|---|---|
| 1. | **ALKALOIDS**<br>Dragendroff's test<br>Wagner's test | **The orange brown precipitate** was observed.<br>**A reddish-brown precipitate** was observed<br>Presence of alkaloids |
| 2. | **AMINO ACIDS**<br>Ninhydrin test | **A purple color** ring is observed<br>Presence of proteins and amino acids |
| 3. | **CARBOHYDRATES**<br>Molisch's test | **A purple color** ring appeared between two layers Presence of carbohydrates |
| 4. | **GLYCOSIDES**<br>Coumarin glycosides<br>Legal's test | **Blue-green fluorescence is** observed. Presence of coumarin glycosides<br>**The pink or red color was not** observed. Absence of cardiac glycosides |
| 5. | **FLAVONOIDS**<br>Shinoda test | **The pink color was not** observed.<br>Absence of flavonoids |
| 6. | **TANNINS**<br>Ferric chloride test | **A deep blue-black color** was observed. Presence of tannins and phenolic compounds |
| 7. | **SAPONINS**<br>Froth formation test | **Persistent foam was** observed. Presence of saponin glycosides |
| 8. | **STEROIDS and TRITERPENOIDS** | **First red, then blue and finally green** color was observed. Presence of steroids |

| | | |
|---|---|---|
| | Liebermann Burchards test | and triterpenoids |

**Table 3. *invitro* Antioxidant activity**

| Absorbance control = 0.4179 | | | | |
|---|---|---|---|---|
| **Concentration** | **Absorbance** | **% Inhibition of DPPH radical** | **Absorbance of standard ascorbic acid** | **%inhibition of DPPH radical of standard ascorbic acid** |
| 2mg/ml | 0.3742 | 10.45 | 0.325 | 22.23 |
| 6mg/ml | 0.3122 | 25.29 | 0.230 | 36.96 |
| 10mg/ml | 0.2500 | 40.17 | 0.200 | 52.14 |

**Table 4 *Invitro* Antidiabetic Activity of *L acidissima* fruit Extract**

| Absorbance control = 0.0519 | | | | |
|---|---|---|---|---|
| Concentration | Absorbance | % Glucose uptake of test | Absorbance of Metronidazole | % Glucose uptake of Metronidazole |
| 2mg/ml | 0.0170 | 67.24% | 0.0320 | 38% |
| 4mg/ml | 0.0126 | 75.72% | 0.0280 | 46% |
| 6mg/ml | 0.0085 | 83.62% | 0.0230 | 55% |
| 8mg/ml | 0.0055 | 91.40% | 0.0519 | 62% |
| 10mg/ml | 0.0006 | 98.84% | 0.0153 | 70.5% |

**Table 5 *invitro* Antimitotic Activity of *L acidissima* fruit Extract**

| S.No | Sample/Control/Standard | Concentration mg/mL | Time | Average growth of seeds |
|---|---|---|---|---|
| 1 | Methanolic extract-1 | 10 | 24 to 72 hours | 1.1±3.8 |
| 2 | Methanolic extract-2 | 20 | 24 to 72 hours | 0.88±0.8 |
| 3 | Methanolic extract-3 | 30 | 24 to 72 hours | 0.35±0.5 |
| 4 | Methanolic extract-4 | 40 | 24 to 72 hours | 0.01±0.2 |
| 5 | Control (Distilled water) | 0 | 24 to 72 hours | 26.5±5 |
| 6 | Standard – Methotrexate | 0.1 | 24 to 72 hours | 0.001±1.0 |

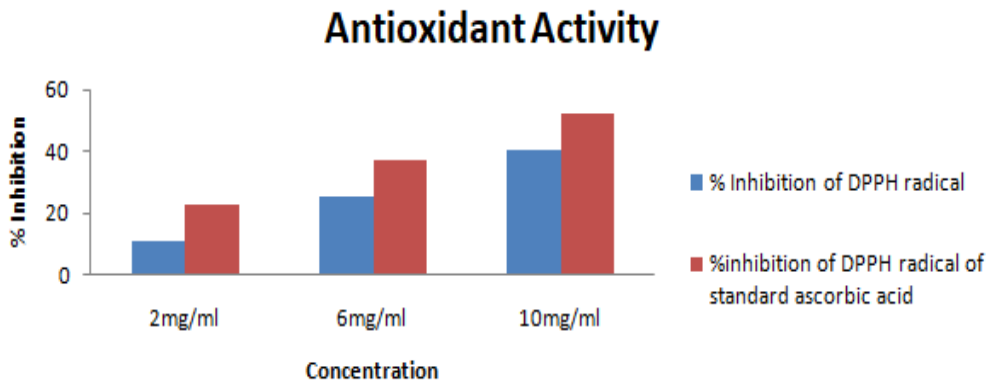


**Figure 1. DPPH scavenging activity of*L acidissima* fruit Extract**

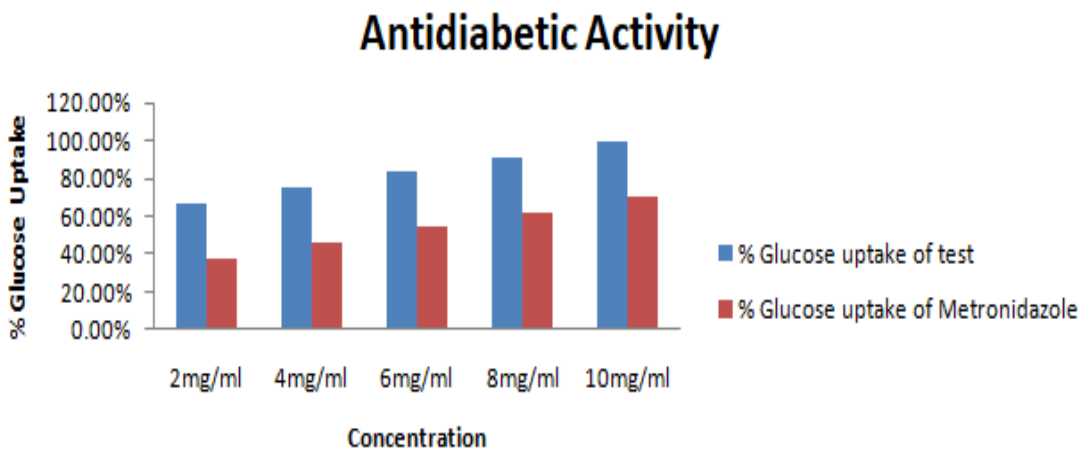


**Figure 2. i*nvitro* Antidiabetic Activity of *L acidissima* fruit Extract**

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**RESEARCH ARTICLE**

# Uncovering Cancer Data Insights: State-of-the-Art Machine Learning Methods for Data Analysis

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**ABSTRACT**

This work explores the use of cutting-edge computational methods to reveal new cancer information. This study uses machine learning as a whole technique to investigate complex patterns and relationships found in cancer datasets. The goal of the investigation is to clarify the fundamental causes of cancer initiation and progression, which may provide important new information for developing treatment approaches in the future. This work aims to improve our comprehension of the intricate dynamics involved in cancer biology by using a data-driven methodology. This work aims to provide a deeper understanding of the condition's landscape by utilizing computational tools to reveal hidden patterns and linkages within cancer data. The overall goal of this research is to use cutting-edge analytical techniques to support continued attempts to decipher the intricacies of cancer biology.

**Keywords :** Machine Learning, Cancer, Analysis, Biomedical, Medical Diagnosis.

## INTRODUCTION

**Introduction to Machine Learning in Cancer Research**

A rare chance to analyze big, complicated datasets in previously unheard-of ways is presented by machine learning. Extensive genomic, transcriptomic, and clinical data might yield important insights for researchers through the use of

complex techniques and computer simulations. This method makes it possible to comprehend the intricacy of cancer on a deeper level, which makes it easier to create treatment programmes that are more individualized and focused.

**Challenges in Traditional Cancer research**

The development of effective medicines for cancer is a huge global health concern due to its complexity and variety. The extensive and complex biological data related to cancer is difficult for traditional approaches to handle. But new developments in computational methods, especially in machine learning, present encouraging chances to decipher the intricate workings of cancer biology.

**Utilizing Advanced Computational Methods**

The modern computational techniques play a major role in cancer research. The goal of this research is to mine the massive volume of cancer information to provide deeper insights by utilizing machine learning. Through the use of these advanced analytical instruments, scientists seek to reveal obscure trends and linkages that are essential to comprehend the genesis of cancer.

**Objectives Study**

Clarifying the basic mechanisms behind the development and spread of cancer is the main objective. The goal of the research is to find novel pharmacological targets, biomarkers of interest and mathematical models of prediction that can help guide clinical judgements. In the end, this research has the potential to completely transform clinical cancer detection, therapy, and management.

**Bridging the Knowledge and Data Gap**

Using computational methods reveals parts of knowledge that were not before known. Through bridging the knowledge gap with data, scientists strive to expedite the creation of more potent cancer treatments and enhance the experience of patients. A new phase in the investigation of the biological origins of cancer has begun with this pluralistic team effort.

## LITERATURE SURVEY

Techniques of machine learning are essential for managing diseases and forecasting cancer outcomes[1,2]. These cutting-edge technologies use sophisticated algorithms to examine enormous volumes of data, including genetic data and health information from patients, spot indications and patterns that can point to the onset or spread of cancer[3]. Researchers and medical practitioners can create prediction models that improve cancer patients' early identification, prognostic evaluation, and customized treatment plans by utilizing machine learning[4]. The imaging sector analysis is one of the main areas where machine learning is used in cancer prediction. Medical pictures, comprising X-rays, magnetic resonance imaging (MRIs), and CT scans, can be analyzed using machine learning algorithms to find tiny anomalies or tumor characteristics that might be signs of cancer[5]. With great accuracy, these algorithms can distinguish between benign as well as malignant lesions, enabling prompt diagnosis and treatment[6]. The analysis of genome-wide data regarding cancer prediction is becoming more and more dependent on machine-learning models. These models can uncover genetic markers linked to particular cancer types or forecast an individual's risk of acquiring cancer by looking at patterns across sequences of genetic material, gene expression stages, ranging, and genetic mutations. Personalized screening plans and focused treatment plans based on each patient's specific genetic profile can be guided by this information[7]. Forecasting techniques for cancer evaluation of risks can be produced by methods such as machine learning that incorporate a variety of clinical data, such as patient demographics, medical histories, test findings, and treatment outcomes. Machine learning can analyze these complex datasets to find risk variables, stratify people with illnesses according to their risk of cancer, and provide guidance for early intervention or measures for prevention that lower the risk of cancer. The creation of predictive models for evaluating the prognosis and response to therapy of cancer is made possible by machine learning techniques[8,9]. These models predict disease progression, malignancy risk, and outcomes in terms of survival for cancer patients by evaluating

longitudinal medical information, including tumor growth dynamics, protocols for treatment, and clinical outcomes[10]. These prognostic models enable medical professionals to plan treatments and give follow-up care with knowledge. By making it easier to identify the best course of action for each patient based on their unique traits and the biology of their tumor, machine learning additionally revolutionized the area of precision oncology[11,12]. Through the integration of clinical data and genetic profiling, machine learning algorithms are able to forecast reactions to treatment possibility and suggest tailored therapeutic treatments that maximize each patient's chances of success, such as targeted medicines or immunotherapies[13]. Predictive analytics is a technique that is powered by machine learning that may enhance patient recruitment and clinical trial design for cancer. Through the examination of past trial information and patient attributes, these algorithms can recognize prognostic biomarkers, categorize patient groups, and refine trial procedures to improve trial outcomes and hasten the creation of innovative cancer treatments[15,16,17]. Additionally essential to the control of population health and cancer surveillance is machine learning. The algorithms for machine learning can prioritize funding for cancer assessment and prevention programs, identify high-risk populations, and provide guidance for health care policy initiatives aimed at lowering the burden of cancer by analyzing population-level medical information, such as cancer occurrence rates, shifts in demographics, and environmental factors[18,19]. The potential for machine learning applications to transform cancer prediction as well as administration is enormous. These applications facilitate the creation of precise, individualized predictive models that improve early detection, prognosis evaluation, treatment choices, and population health approaches[20]. The potential for these technologies to improve results in cancer and change the oncology care landscape is enormous as they develop further[21,22].

To help with the early identification, prognosis, and individualized therapy of cancer, machine learning (ML) algorithms have shown great promise in the analysis of complex scientific information, including genomic information, imaging, and clinical data. ML models can detect subtle patterns and indications indicative of the existence of cancer, the categorization of the cancer subtype, and the prognosis of the patient by utilizing large-scale datasets and sophisticated analytics[23]. Predictive models driven by machine learning (ML) can also help oncologists choose the best course of treatment, forecast how well a treatment will work, and find possible targets for therapeutics for precision medicine strategies. Additionally, machine learning algorithms can aid in the creation of novel risk prediction models, treatment approaches, and screening techniques, all of which can enhance patient outcomes and lower cancer death rates[24,25].

## METHODOLOGY

**Role of Supervised Algorithms in Cancer Classification**

Using characteristics like radius_mean, texture_mean, and perimeter_mean, machine learning techniques including Random Forest and Support Vector Machines (SVM) are essential for analyzing cancer data. These algorithms aid in the classification of tumors as benign or malignant, which facilitates efficient assessment and planning for treatments. These methods help personalized therapy better comprehend cancer uniqueness and improve patient satisfaction.

**Support Vector Machine for Tumor Classification**

SVM is an effective classification algorithm that divides observations into different categories by identifying the best hyperplane. It works especially well with statistics that have distinct class borders and high dimensions. Based on characteristics including radius, substance, and border, SVM can identify cancerous or benign tumors in study studies.

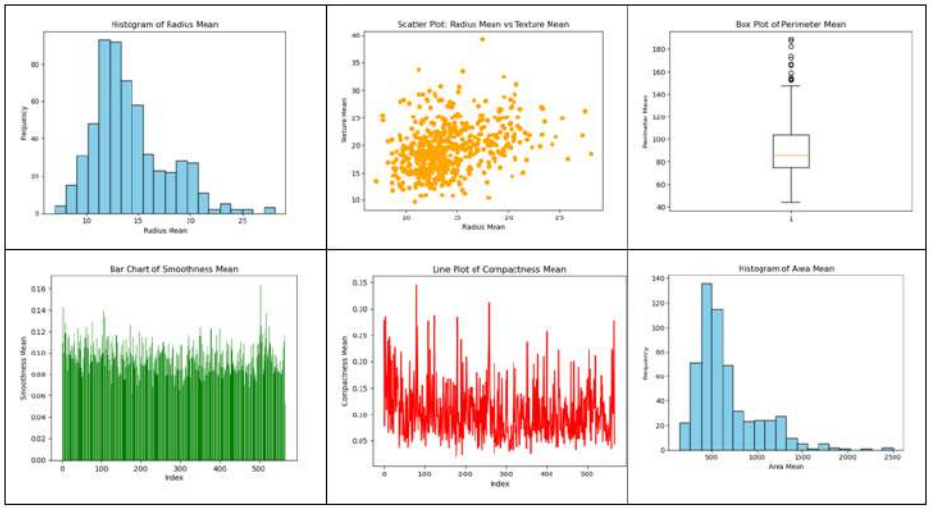
**Random Forest and Its Advantages**

Random Forest makes forecasts by aggregating the outcome results of many decision-making trees. This approach helps find key characteristics for cancer detection by effectively handling data with numerous dimensions, preventing excess fitting, and providing significance of features. Because of its durability, it can be used to process cancer data and increase the accuracy of categorization.

**Hierarchical Clustering in Cancer Analysis**

Every single point of information is initially clustered separately in hierarchical clustering, which then merges the clusters according to characteristics to form a structure resembling a tree. By considering the properties of the tumors, it creates a diagram called a dendrogram in disease data analysis. Understanding tumor behavior and developing targeted treatments are aided by the identification of subgroups with patterns that may hold clinical importance.

**Data Analysis with the Different Features**




## RESULTS

SVM and Random Forest, two supervised learning methods, performed admirably. Recall and pinpoint accuracy ratings added to their effectiveness in accurately classifying cancer cases. Unsupervised methods like K-means clustering and hierarchical clustering provided valuable insights into the underlying structure of the cancer dataset, which facilitated exploratory research.

**Results for Supervised Algorithms**

```
SVM Classifier:
Accuracy: 0.956140350877193
Precision: 0.975
Recall: 0.9069767441860465

Random Forest Classifier:
Accuracy: 0.9649122807017544
Precision: 0.975609756097561
Recall: 0.9302325581395349
```

## CONCLUSION

This work applies state-of-the-art computational techniques to investigate the biology of cancer, using machine learning to interpret complex patterns in datasets. With the potential to guide future therapeutic approaches, the research attempts to identify the underlying causes of cancer development and progression. Through the use of data-driven methodology, it aims to improve comprehension of cancer dynamics. Computational methods help reveal patterns concealed in cancer data, offering new perspectives on the disease landscape. The ultimate objective is to

expand our understanding of cancer biology through the application of cutting-edge analytical tools, opening the door to more effective treatments and diagnostics.

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**RESEARCH ARTICLE**

# Production, Optimization and Partial Purification of Amylase from the *Bacillus megaterium* for the Removal of Biofilm

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## ABSTRACT

Biofilm is a group of microorganisms living with a self-produced protecting layer, extracellular polysaccharides (EPS). Many pathogens are EPS producing and biofilm forming organisms, due to this the organisms also show the resistant towards the antibiotics. Our study had been focused on the green method (enzymatic method) to remove the biofilm formation. Among the various enzyme, Amylase enzyme is the most applicable enzyme to remove the biofilm formation. Amylase is a valuable enzyme with significant applications. In the current work, three different *Bacillus* species were selected for the production of amylase enzyme. On the basis of the primary and secondary screening, *Bacillus megaterium* gave the highest production of amylase enzyme and was selected for the further statistical optimization and partial purification. After the statistical optimization 5.4-fold amylase activity was increased. SDS PAGE shows that after the partial purification of the amylase enzyme, the molecular weight of the amylase enzyme extracted from *Bacillus megaterium* is 20KDa. The partial purified enzyme was successfully removed the biofilm of *Escherichia coli* (79.94%), *Pseudomonas aeruginosa* (70.68%), *Staphylococcus aureus* (57.07%). The biofilm removal was further confirmed by light microscopy and fluorescence microscopy.

**Keywords:** Biofilm, Amylase, Bacillus spp. Production and Biofilm removal

## INTRODUCTION

There is an accumulation of sessile microbial species which is linked biotic or abiotic surface it is known as Biofilm. "A 3D polymer matrix" also have been found on the liquid surfaces. Biofilm forming bacteria survive under the harsh environments like depletion of nutrition, exposure of heat radiation, antibiotic and other stress factors due to the production of sticky gum like substance known as exopolymer substance (EPS). Biofilm consists of intricate molecules such as proteins, polysaccharides, nucleic acid, eDNA, glycoproteins, and lipids. This EPS provides benefits to the biofilm bacteria like attachment on abiotic and biotic surfaces aggregation as a cluster form and main in biofilm formation. The examples of Biotic and Abiotic surface are human tissues, organ and catheter, surgical instrument respectively [1]. Around 90% of biofilm mass is made up of EPS (Extracellular Polymer Substance). The biofilm forming bacteria also present on medical devices and they form biofilm on such devices. Almost 80% of diseases can be due to biofilm-associated pathogens. Basic components play a vital part in biofilm arrangement. EPS makes a difference ensure bacterial cells from anti-microbials, making them safer to the medicate [2]. Due to this it become a major concern for the medical settings these because these organisms show a resistant toward the antibiotic and it became a for major hurdle for the treatment. Amylase enzymes are derived from various sources such as plants, animals, and microorganisms. Among these, microbial amylases are the most widely produced and utilized in industries owing to their exceptional productivity and ability to withstand high temperatures [3]. Microbial enzymes have grown more and more popular because they are easy to isolate in high quantities, can be produced 76 easily and inexpensively, and are stable in a variety of difficult environments [4]. The primary advantages of using bacteria in amylase production are the inexpensive techniques for mass-producing amylases and the simplicity with which they may be modified to produce enzymes with specific characteristics [4]. The EPS can be broken down and bacteria can be killed by the enzyme amylase, which inhibits the production of biofilms [2].

In our study, we mainly focused on removal of the biofilm using a green/ natural method, i.e., enzymatic method. In this study we are focus on production of Amylase enzyme from *Bacillus megaterium* spp. Optimize the Single factor to enhance the amylase activity, partial purification. The partially purified amylase enzyme is used as an antibiofilm compound to remove biofilm.

## MATERIALS AND METHOD

All the chemicals including iodine, 3-5-Dinitro salicylic acid (DNS), Soluble Starch, Yeast Extract, $CaCl_2$, Peptone, $KH_2PO_4$, $MgSO_4$, $FeSO_4$, $K_2HPO_4$, Beef extract, NaCl, Acrylamide, Bisacrylamide, Hydrochloric acid (HCl), Ammonium persulphate, Glycine, Glycerol, β mercaptoethanol, Tetramethyl ethylenediamine (TEMED),Coomassie Brilliant Blue- R 250, Methanol, Acetic acid, Glacial acetic acid, Crystal violate, Isopropyl Alcohol, ethidium bromide (EtBr) are grade of highest purity available procured from Hi-Media Laboratories, India. *Bacillus subtilis* ATCC, *Bacillus megaterium* ATCC, *Bacillus cereus* ATCC were obtained from the American Type Culture Collection (ATCC).

**Primary Screening**

The primary screening was used to identify the ability of the organisms for the production of amylase enzyme. Three different *Bacillus spp*. (*Bacillus subtilis* ATCC, *Bacillus megaterium* ATCC, *Bacillus cereus* ATCC) were screened for the amylase production. The composition of the primary starch agar medium was: Soluble starch (10 gm/l), Peptone (5 gm/l), Beef extract 100 (5 gm/l), NaCl (2 gm/l) $MgSO_4$ (2 gm/l), Agar (10 gm/l). Organisms were inoculated on the primary starch agar medium and incubate at 37°C for 24 hours. Next day, all the incubated plates were flooded with the iodine and based on the hydrolysis of starch, the zone of clearance 103 was observed and recorded.

**Secondary Screening**

The result of the primary screening was further confirmed by the secondary screening. starch liquid media (Soluble starch-10 gm/l, Peptone-5 gm/l, Beef extract-5 gm/l, NaCl-2 gm/l, 107 $MgSO_4$-2 gm/l) was used for the secondary screening. *Bacillus subtilis* ATCC, *Bacillus megaterium* ATCC, *Bacillus cereus* ATCC was inoculated into the starch broth

medium and incubated at 37ºC for overnight. Next day for the enzyme assay, enzyme was extracted by the centrifugation at 7000 rpm for 10 minutes. Supernatant was collected for the amylase assay.

**Amylase Assay:**

Miller et.al., 2002 illustrate the method for amylase assay to determine the amount of reducing sugar released from soluble starch. The activity of amylase enzyme obtained from bacterial cell was evaluated by 3,5-dinitrosalicylic acid (DNS). The samples were taken (500 µl of enzyme from supernatant + 500 µl of substrate 1% starch + 1 ml of $PO_4$ buffer) and incubate 116 37ºC for 10 minutes. 1 ml of freshly prepared DNSA solution were added to stop the reaction. Tubes were placed in water bath at 100ºC for 10 minutes. Cool it down under running tap water and add 10 ml of Distilled water. Absorbance was taken at 540 nm using a Digital colorimeter (Advance lab equipment). A glucose standard curve was created to determine the concentration of glucose produced in each solution by plotting absorbance at 540 nm against the amount of glucose released. Amylase activity was then calculated, one unit of enzymatic activity is equivalent to the amount of the enzyme needed to catalase the synthesis of reducing sugar under typical assay circumstances, which is equal to 1µmol/min of glucose (Abo-Kamer et al., 2023)[4].

The following equation was applied for the estimation of enzyme activity

$$Enzyme\ Activity = Amount\ of\ reducing\ sugar\ \times \frac{1000}{molecular} weight\ of\ glucose\ \times time$$

**Optimization of different parameters for Amylase production under submerge fermentation (single factorial):**

**Effect of moistening medium for amylase production:**

The selection of moistening medium is a very important parameter for the maximum production of Amylase enzyme. The six different moistening media were screened for the amylase enzyme Production [5,4].

Media T1: (gm/l). Glucose-20; yeast extract-10; $MgSO_4.7H_2O$-1; $KH_2PO_4$- 2.

Media T2: (gm/l). Peptone-10; starch-10; yeast extract- 20; $CaCl_2$-0.05; $MgSO_4$-0.25; 139 $KH_2PO_4$- 0.25; $(NH_4)2SO_4$, -0.25; $FeSO_4$-0.01.

Media T3: (gm/l). Soluble starch-10; peptone-6; MgSO4-0.5; potassium chloride-0.5.

Media T4: (gm/l). Soluble starch-20; yeast extract-5; magnesium sulphate-1; $CaCl_2.2H_2O$-0.2; NaCl-1.

Media T5: (gm/l). Soluble starch-10; peptone-25; $KH_2PO_4.3H_2O$-1.5; $NaSO_4$-1.5; $FeSO_4.7H_2O$-0.03; $MnCl_2.4H_2O$-0.15; CaCl2.2H2O-0.45.

Media T6: (gm/l). Soluble starch-5; glucose-7.5; lactose-5; Yeast extract-5; Peptone-12.5; Ammonium sulphate-12.5; Magnesium sulphate-0.75.

The Submerged fermentation was done for the production of amylase by inoculating culture 148 into each flask and incubated at 37°C for overnight. Next day, enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and check enzyme activity by DNS method.

**Effect of inoculum Size for amylase production**

The effect of the inoculum size on the enzyme production is another important parameter. The production medium was inoculated with various inoculation size, ranging from 200 µl to 1600 µl. After the inoculation, flasks were incubated at 37°C for overnight. Next day, enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and DNS method was performed to check the enzyme activity.

**Effect of time period on amylase production**

The evaluation of effect of time on the amylase enzyme was determine by incubating the production media for different interval. The fermentation media was incubated at (0,24,48, 96,72,120 hours). Next day, enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and enzyme activity check by DNS method [6].

**Effect of Temperature for amylase enzyme production**

Temperature is one of the important factor for submerged fermentation, because enzyme and bacteria both sensitive to temperature. The effect of temperature on amylase enzyme period was examine by incubated flasks at different temperature (15,27,37,72,96 ºC). Next day enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were 167 collected and DNS method was performed to check the enzyme activity [7].

**Effect of pH on amylase production**

The effect of different pH of the production media on amylase activity was examined. The pH of the production media was assessed in the range of pH1- pH9. Next day, enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and enzyme activity check by DNS method [7].

**Effect of various Carbon sources on amylase production**

Several carbon sources were used for amylase enzyme production. Carbon sources such as 175 starch, glucose, sucrose, mannitol, lactose was incorporated into the production medium. After 176 the inoculation, flasks were incubated at 37°C for overnight. Next day, enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and enzyme activity analysed by DNS method [7].

**Effect of various Nitrogen sources on amylase production:**

The effect of various nitrogen sources on amylase enzyme production was evaluated. Nitrogen sources such as yeast extract, peptone, beef extract, ammonium sulphate were used in this experiment. After the inoculation, flasks were incubated at 37°C for overnight. Next day, enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and DNS method was performed to check the enzyme activity [8,4].

**Multi factorial experiments for optimization of amylase production:**

The optimization of the production media for amylase enzyme was done in three step techniques. In the first step Plackett-Burman Design (PBD) was used to examine the relative important components of a media affecting the amylase enzyme activity. Most important components of the PBD were further calculated for their ideal level with the help of Central Composite Design (CCD) and contour plots. In the last step computational statistical analysis (ANOVA) to validate the model's fitness as measured by the determination of coefficient (R2).

**Plackett–Burman Design for primary factors (PBD):**

Plakett-Burman design is a mathematical compute design and it is used to evaluate the significance of 5 different medium components of the production media for the production of amylase. Different experimental combinations were executed to minimize unrelated variables that affect amylase production. Five different medium components (Starch, peptone, $MgSO_4$, $CaCl_2$, NaCl) were screened out in the combination of 8 according to the Plackett- Burman Design by using Design expert 13 software. In the Plackett-Burman Design, each variable being represented at low level (-) and high level (+) including two dummy variables. The following equation was used to calculate the effect of each variable.

$$E(xi) = \frac{2(\Sigma M^{+} - M^{-})}{N} \qquad \text{eq.(1)}$$

Where, E(xi) = the concentrated effect of the tested variable, $M^{+}$ and $M^{-}$ = amylase production from the trials where the variable (xi) was present at high and low concentrations, respectively and N= number of trials. The experimental error was evaluated by calculating the variance among the dummy variables as follow:

$$V_{eff} = \Sigma(E_d)^2/n \qquad \text{eq.(2)}$$

Where, Veff = concentration effects of variance, $Ed$ = concentration effect of the dummy variable and n = number of the dummy variables. The standard error of the concentration effect was a square root of variance of an effect and the significance level (p-value) of each concentration effect was evaluated using the student's t-test:

$$t_{(xi)} = E_{\frac{(xi)}{SE}} \qquad \text{eq.(3)}$$

Where, E(xi) is the effect of variable xi.

**Central Composite Design:**

Response surface methodology (RSM) represents a widely acknowledged statistical model that used to optimized and evaluation of the impact of different components of production medium and their interaction. Design expert 13 software was used to analyse and response surface graph. CCD analysis was done on the independent factors that showed a substantial impact on amylase activity by PBD. Three variables (Starch, $MgSO_4$, $CaCl_2$) were used. In CCD, 3D response curves were plotted against any two independent parameters, with the third independent factor maintained constant or at the 0 level, to examine the optimum values and the combined influence of the selected variable on amylase production. ANOVA was use to assess the significance of model and the regression coefficients A regression equation was used to examine the quality of the second order polynomial equation.

$$Y=\beta 0+\Sigma\beta ixi+\Sigma\beta ijxixj+\Sigma\beta iixi2 \qquad \text{eq.(4)}$$

Where, Y= predicted response, β0= offset item, βi= linear offset coefficient, βii = squared offset coefficient, βij = interaction effect and xi = the dimensionless code of the Xi.[19].

**Comparison of Unoptimized and Optimized media:**

The comparison of unoptimized media and statistical optimized and was done to study the increase in fold production of amylase enzyme. Culture was inoculated in both the unoptimized and statistically optimized medium. After the inoculation, flasks were incubated at 37°C for overnight. Next day enzyme was extracted by centrifugation at 7000 rpm for 10 minutes. Supernatant were collected and enzyme activity analysed by DNS method.

**Partial Purification:**

**Ammonium sulphate precipitation and dialysis**

Two step purification was done for the amylase enzyme produced by the *Bacillus megaterium* ATCC. The crude amylase enzyme that was produced during the immerse fermentation was centrifuged for 10 minutes at 7000 rpm (4°C). The complete protein was precipitated using the ammonium sulphate supernatant after centrifugation. The range of 0-90% saturation was used for the ammonium sulphate precipitation process. To extract the salts from the fraction, dialysis was carried out following by the ammonium precipitation method. The active fraction with highest amylase activity was dialyzed against the phosphate buffer (pH-7.0) for 6 hours.

**Enzyme**

**Enzyme molecular weight determination by SDS – PAGE:**

High enzymatic activity fractions was obtained by partial purification. Protein assay was done by Folin lowry's method [9]. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 12% gel was then used to determine relative molecular mass [10]. The gel was stained with the help of Coomassie brilliant blue R-250 dye and its molecular mass marker ranged from 6.5 to 200 kDa. The standard protein marker contain (Myosin pig- 200,000 Da), (β-galactosidase E.*Coli* - 116,000 Da), (Phosphorylase B Rabbit muscle- 97,200 Da), (Serum 250 Albumin Bovine- 66,409 Da), (Ovalbumin Hen egg White-44, Da), (Carbonic anhydrase Bovine- 29,000 Da), (Trypsin inhibitor Soyabean- 20,100 Da), (Lysozyme Hen egg white- 14,300 Da), (Aprotinin Bovine pancreas- 6,500 Da).

**Biofilm Removal:**

***Biofilm removal by plate assay:***

A biofilms of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus* was developed on 96-well polystyrene microtiter plates. The process of biofilm development includes the dilution of overnight grown cultures (*E. coli, S. aureus, and P. aeruginosa*) in LB broth to an absorbance at 600 nm of 0.01 (1 x 108 CFU/mL). The diluted 200 µL of cell suspension was applied to each well on a 96-well polystyrene microtiter plate. The concentration (80 µl, 100 µl and 150 µl) of the partially purified amylase enzyme (at the time of biofilm formation and after the formation of the biofilm) and time period (12-48 hours) was optimized to remove the biofilm. All the microtiter plates were incubated at 37ºC. Once the incubation period was completed, the planktonic cells of the biofilm was removed using the 10 mM phosphate buffer saline (PBS). The adherent cells of the biofilms were stained with the 0.2% of crystal violet for 10 minutes. After 10 minutes of exposure of crystal violet, the biofilm of the adherent bacterial cells was cleaned three times with the help of sterile distilled water to get rid of any remaining colours. Finally, the plates were destained with Isopropyl Alcohol (IPA). The production of the biofilm by the organism and removal of the biofilm by partially purified amylase enzyme was evaluated by taking absorbance at 595 nm using (Thermo scientific Multiscan go-nanodrop) [11,12]

$$\text{Percentage of biofilm inibition} = \frac{\text{Optical density of control sample} - \text{optical density of test sample}}{\text{optical desity of control sample}} \times 100$$

**Microscopic techniques:**

**Anti-biofilm Activity by light Microscopy**

The biofilm formation capability and the removal of biofilm by partially purified amylase enzyme was evaluated by light microscopic technique. Prepare a smear of culture (*E. coli)* on glass slide and allow it to air dry. The smear was treated with the 150 µl partially purified 280 amylase enzyme. The glass slide was incubated for 48 hours at 37ºC. Next day, the glass slide was stained with 0.2% of crystal violet. The excess dye was removed by washing with distilled water and visualized under a light microscope (40X) [13].

**Anti-biofilm Activity by fluorescent Microscopy:**

The biofilm formation capability was also examined by fluorescent Microscopy. Prepare a 285 culture smear on glass slide allow it to air dry. The smear was treated with the 150 µl partially 286 purified amylase enzyme. The glass slide was incubated for 48 hours at 37ºC. Next day, the glass slide was stained with ethidium bromide (EtBr) dye. The excess dye was removed by washing with distilled water and visualized under a fluorescent microscope Nikon ECLIPSE 289 Ti2.

## RESULTS AND DISCUSSION

**Primary Screening**

In the present study, three different *Bacillus spp*. (*Bacillus subtilis* ATCC, *Bacillus megaterium* ATCC, *Bacillus cereus* ATCC) were used for screening of amylase production. All the three species of *Bacillus* i.e. *Bacillus subtilis* ATCC, *Bacillus megaterium* ATCC, *Bacillus cereus* ATCC exhibited positive results, clear zone on 1% starch agar plate. Figure 1 shows that the highest zone of hydrolysis was obtained by *Bacillus megaterium* ATCC (16 mm) on 1% starch agar plate. This result indicates that *Bacillus megaterium* ATCC has the highest ability to produce the amylase. *Bacillus subtilis* ATCC, *Bacillus cereus* ATCC shows zone of hydrolysis (13 mm, 12 mm) respectively. Similar results were observed by the [14]. They observed that 300 *Bacillus* spp. showed zone of hydrolysis in the range of 10- and 17-mm. Similar result was also recorded by abo-kamer et al., 2023 [4] that zone of hydrolysis was 15 mm by *Bacillus subtilis*.

**Secondary Screening**

Secondary screening method is used to confirm the results of primary screening. All the three different species of *Bacillus, Bacillus subtilis* ATCC, *Bacillus megaterium* ATCC, *Bacillus cereus* ATCC shows positive results in the primary

screening, hence all three species were 306 selected for the secondary screening. Secondary screening was done by the production of amylase enzyme under submerged fermentation. On the basis of the secondary screening, **Figure 2** represents the *Bacillus megaterium* ATCC shows the highest amylase enzyme activity (564.10 U/ml) however *Bacillus cereus* ATCC had the lowest enzyme activity (351.282 U/ml). The activity of the amylase enzyme shows the ability of the organism to break down starch into glucose by producing amylase enzyme, so higher the enzyme activity higher the amylase enzyme production by the organism. abo-kamer et al. 2023[4] reported that *Bacillus cereus* produced 135 U/ml amylase enzyme.

**Optimization of different parameters for Amylase production under submerge fermentation:**

**Effect of moistening medium for amylase production:**

One of the main factors in submerged fermentation that can be effectively optimized for maximum amylase production is the selection of moistening medium. The experiment was conducted using six different media (T1-T6). Among all the six different media, T4 media was the most suitable media for the production of amylase enzyme (1188.03 U/ml) shows in **Figure 3a.** Hence Optimum production was related to the existence of additive nutrients and metal ions like $MgSO_4$, $CaCl_2$, NaCl which are the essential growth of the *Bacillus megaterium* ATCC bacteria. Optimization of the moistening medium varies with the species of the microorganisms [4]. Starch, $MgSO_4$, CaCl2, NaCl provides essential support for bacterial growth, enzyme function and stability. Together these components create a conductive environment for bacterial growth and efficient enzyme production.

***Effect of inoculum Size for amylase production***

Another important factor is inoculum size. The inoculum size was optimized by inoculating inoculum in the range of the (200µl- 1600µl). Figure 3b represents that the amylase activity was very less at lower inoculum size, as the inoculum size increased the enzyme activity was also increased. The highest enzyme activity was obtained at 1000µl (897.43 U/ml). Further 332 increase in the inoculum size, shows a negative effect on the amylase activity.

**Effect of various time periods on amylase production**

Optimization of time plays a vital role for the production of amylase enzyme. The experiment was conducted at different time intervals (0 - 120 hours). According to **Figure 3c** the highest production of amylase was obtained at 24 hours (1068.38 U/ml). However further incubation leads to the decreasing enzyme activity and at the 120 hours (68.37U/ml) amylase activity was obtained. Rakaz et al., (2021) [15] reported the highest activity for *Bacillus cereus* 0.122 U/min/ml at 24 hours, it is very less as compare to our result they also reported that incubation time increased enzyme activity decreased.

**Effect of various Temperature for amylase production**

The production of amylase enzyme is significantly affected by the optimization of temperature. During the fermentation process there is a rise in temperature due to the respiration of organisms (Darabzadeh et al., 2019) [16]. Figure 3d shows the effect of temperature on amylase production by *Bacillus megaterium* ATCC. In the current work, the result shows that there is an increase in the amylase activity with the increasing temperature and maximum amylase production was achieved at 37ºC (897.43 U/ml). However, further increase in temperature 348 decreases enzyme activity. These findings show that above the 37º C did not support the growth of microorganisms as well as the production of amylase. Similar results finding by Sobhy et al., (2023) [7] amylase output 857.92 U/ml at 37ºC.

**Effect of various pH for amylase production**

Another important factor amylase production is pH of the production media. **Figure 3e** illustrates the effect of pH on amylase activity. The maximum production of amylase enzyme was obtained at pH 7 (1141.81 U/ml). Similar findings were reported by Olajuyigbe and for Ajele (2005) [17], they found that *Bacillus* species require an ideal pH of 8.0(60%). Sobhy et al., (2023) [7] also find optimum pH is 7 (856.92 U) for the production of amylase enzyme by 358 *Bacillus* spp.

**Effect of various carbon source for amylase production**

Carbon sources play a vital role in fermentation as well as play a key role in cellular growth of the microorganism. In the current study, five different carbon sources (Starch, Glucose, Sucrose, Mannitole and Lactose) were selected to produce the amylase enzyme from *Bacillus megaterium* ATCC. The figure 3f shows the effect of different carbon sources on amylase production. Among the five carbon sources, maximum amylase production. (1111.11 U/ml) was obtained by using starch as a carbon source. Similar result was reported that carbon source is suitable for amylase production from *Bacillus aryabhattai* KIIT BE-1 by Ojha et al., 2020 367 [18].

**Effect of various nitrogen source for amylase production**

Nitrogen compounds are also important at the time of the fermentation by microorganisms. The effect of different nitrogen sources on the production of amylase enzyme is shown in Figure 3g. Among the four nitrogen sources, Peptone gave the highest production of amylase enzyme (1247.86 U/ml). Similar result reported by Sobhy et al., (2023) [7]. They showed that peptone as a nitrogen source most suitable for the amylase production (878.45U) from streptomycin.

**Multi factorial experiments for optimization of amylase production:**

Plackett-Burman Design has been used to analyse the components that are necessary for the production medium to produce amylase from *B. megaterium* ATCC. The high (+) and low (-)concentrations of the independent variables for the optimization research were selected. In the Plackett-Burman Design, the equivalent amylase production in terms of U/ml for each of the two levels of concentration for each medium component over 8 trials. D1–D2 represents the dummy variables, and the variables designated as X1–X5 indicate the components of the moistening medium. Based on the amylase production (unit per millilitre), Table 1 display the effect, t (xi), p-value, standard error, and confidence level for each medium component. The effects of the different components of the moistening medium were screened out with a confidence level higher than 95%. The CI level of Starch, $MgSO_4$, $CaCl_2$ is 98.81%, 99.96% and 99.56% respectively. Among the five medium components, these three components they were significant variables. However medium components were seen to be less significant variables. According to Placket-Burman design, the medium component had a negative effect, meaning that a lower concentration was needed than the low (-) concentration that was given. However positive effects so the medium component that the concentration of composition needed to be higher than the plackett-Burman design high (+) value [19].

**Central Composite Design**

Fisher's statistical test for Analysis of variance (ANOVA) was done for the response of amylase production. In placket- Burman design the variable of moistening medium showing 396 positive and negative effects with or above at 95% confidence level were further optimized by Central Composite Design (CCD). Total 20 experiments were run with three variables (Starch, $MgSO_4$, $CaCl_2$) using different concentrations. With the help of quadratic multiple regression to describe the relationship between the dependent and independent variables, the Design expert program 13 produced the following equation in terms of coded factors.

$$Y = 9992.27 + 831.12A + 711.53B - 451.28C + 280.87\ AB - 1223.40AC + 473.18BC - 1071.18\ A^2 - 1252.64B^2 - 1252.64C^2 \qquad eq(5)$$

**Table 2** represents the results of the analysis of variance (ANOVA) used to validate the 404 regression coefficient, which shows that the model was significant. According to the analysis of variance, the quadratic model was not significant with a lack fit value of 0.096, a model value of 3.53, and p > f indication. Thus, there was high correlation between the predicted and experimental results for analysis. According to the analysis, A2, B2, and C2 p- value are all less than 0.05, indicating significance. Since each component's P value is less than 0.1, the model was significant. A factor level region association between a variable's response and its experiment of a given variable is another benefit of using 3-D graphs. Two different medium components at different concentrations are shown in the 3 D plot, while the other medium component The Design Expert software was used to examine the synthesis of amylase using

3-D plots. l level is displayed using a 3-D plot and contour plot. Assuming the ideal concentration are kept at a fixed concentration. The interaction between $MgSO_4$ and starch is shown in Figure 4a. The 3-D graphic demonstrates that when the concentrations of starch and $MgSO_4$ were very low, there was less amylase production. When the concentrations of starch and $MgSO_4$ increased simultaneously, the production of the enzyme amylase increased. The highest amount of amylase enzyme was obtained at the concentration 18 g/l of starch and 0.9 g/l of $MgSO_4$. Further increased in the concentration of starch and $MgSO_4$ there was a decrease in amylase activity. The findings indicate that while starch and $MgSO_4$ alone acted as non-significant components, together had a synergistic effect on the enzyme's ability to produce amylase.

**Figure 4b** illustrates the correlation of $CaCl_2$ and starch for amylase production. Figure shows that at the lowest concentration of starch and $CaCl_2$, the production of amylase is very low. Amylase was synthesized at a higher rate when the concentration of starch and $CaCl_2$ were increased simultaneously. The highest amount of amylase enzyme produced at the concentration of 18 g/l of starch and 0.39 g/l of $CaCl_2$. This finding indicates that starch and $CaCl_2$ individually acted as non-significant components but they had a synergistic effect on the production of the enzyme amylase.

Figure 4c illustrates the interaction between $CaCl_2$ and $MgSO_4$. The three-dimensional graphic indicates that amylase production was lower at very low concentrations of $MgSO_4$ and $CaCl_2$. When $MgSO_4$ and $CaCl_2$ concentrations increased at the same time, the production of the enzyme amylase also increased. The highest amount of amylase enzyme produced at the concentration of 0.9 g/l of $MgSO_4$ and 0.39 g/l of $CaCl_2$. Further increase in the concentration of $MgSO_4$ and $CaCl_2$ lead to decrease in the amylase enzyme activity. The findings indicate that $CaCl_2$ and $MgSO_4$ individually acted as non-significant components, but both the components showed a synergistic effect on the synthesis of the enzyme amylase.

The actual value v/s predicted value for the production of amylase enzyme by *Bacillus megaterium* ATCC was evaluated using model equation (5). The result shows that the most of the points were located near the adjustment line which indicates that the experimental value were similar to the predicted values by the model. The predicted concentration of three different variables were starch 18 g/l, $MgSO_4$, 0.9 g/l and $CaCl_2$ 0.39 g/l with predicted highest amylase enzyme production of enzyme activity statistically optimized (8974.16 U/ml).

**Partial Purification:**

**Ammonium sulphate precipitation and dialysis**

The extracellular amylase enzyme was partially purified by the ammonium sulphate precipitation followed by dialysis. The ammonium sulphate was used to saturate the crude enzyme from 0 to 80%. The specific activity of amylase enzyme increase with the concentration of ammonium sulphate in the fraction of precipitate and decreased in the fraction of supernatant. The highest specific activity (127.4 U/mg) was recorded at 60% saturation level of ammonium sulphate. After the ammonium sulphate precipitation and dialysis of amylase enzyme, 124.7 U/mg and 148.5 U/mg specific activity with 1.28 and 1.53-fold purification was 454 obtained (Table 3).

Liaquat et al., (2015) [20] reported that, $\alpha$-Amylase enzyme from *Bacillus subtilis* (RAS-1) was partially purified with 70% saturation level of ammonium sulphate precipitation. The recovery and purification of amylase from *Bacillus subtilis* (RAS-1) were 37.5% and 2.60. While $\alpha$-Amylase enzyme from *Clostridium perfringens* (RAS-4) was partially purified with 60% of ammonium sulphate precipitation. It exhibited specific activity of 6.01 U/mg, 2.29 purification fold with 32.38% recovery.

**Enzyme molecular weight determination by SDS – PAGE**

The molecular weight of partially purified amylase enzyme from *Bacillus megaterium* ATCC was evaluated by SDS - PAGE. The estimated molecular weight of amylase enzyme was 20 kDa. The contradictory results were reported by Liaquat et.al (2015) [20] and Haq et.al (2010) [21]. They reported that the molecular weight of alpha amylase from *Bacillus subtills* (RAS-1) and *Bacillus subtills* was 52 kDa and 55 kDa respectively.

**Biofilm Removal**

***Biofilm removal by plate assay***

**Figure 5** shows the effect of biofilm removal by the treatment of partially purified amylase enzyme from *Bacillus megaterium* ATCC. The partially purified amylase enzyme was used to remove the biofilm of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* The result shows that amylase enzyme successfully removed 79.94%, 70.68%, 57.07% biofilm of *E. coli, P. aeruginosa, S. aureus* after the 48 hours of treatment with 150 µl concentration respectively. The variation in the removal of biofilm of *E. coli, P. aeruginosa, S. aureus* by amylase enzyme may be due to the variation in the EPS composition Solihin et.al (2021) [22]. Abo-kamer et.al (2023) [4] reported approximately 84% biofilm removal of Pseudomonas *aeruginosa* by purified amylase enzyme from *Bacillus cereus*. Vaikundamoorthy et.al (2018) [3] reported that analyse enzyme produced from the *Bacillus ceruse*, successfully remove the biofilm of *P. aeruginosa* and *S. aureus.*

**Microscopic techniques:**

**Anti-biofilm Activity by light Microscopy:**

The formation of biofilm by *Escherichia coli* and the removal of biofilm by partially purified amylase enzyme was examined by light microscopic technique. The results of the light microscope give the evidence of the successfully removal of biofilm after the treatment of partially purified amylase enzyme from *Bacillus megaterium* ATCC. Figure 6a shows positive control of biofilm formation by *E. Coli* and Figure 6b shows the treated biofilm by amylase enzyme.

**Anti-biofilm Activity by fluorescent Microscopy**

Fluorescent Microscopy was used to study the formation of biofilm by microorganisms and the removal of biofilm by partially purified amylase enzyme from *Bacillus megaterium* ATCC *Escherichia coli* was used as a positive control. The fluorescence microscopic images of fluorescent microscope Nikon ECLIPSE Ti2 shows that after applying the partially purified amylase enzyme there was a less formation of biofilm as compare to positive control. Figure 7a shows positive control of biofilm formation by *E. Coli* and Figure 7b shows the removal of biofilm after treating with the amylase enzyme.

## CONCLUSION

In the present study, three different species of *Bacillus* were selected (*Bacillus subtilis* ATCC, *Bacillus cereus* ATCC and *Bacillus megaterium* ATCC) for the production of amylase enzyme. The results of primary and secondary screening show that *B. megaterium ATCC* gave maximum zone of inhibition (16 mm) and highest enzyme activity (564.10 U/ml). On the basis of this results *Bacillus megaterium* ATCC was selected for the further study. Single factorial and statistical optimization were done to enhance the amylase enzyme production. Statistical optimization gave 5.4-fold increase in the amylase enzyme production by the *B. megaterium* ATCC. After the ammonium sulphate precipitation and dialysis of amylase enzyme, 124.7 U/mg and 148.5 U/mg specific activity with 1.28 and 1.53 fold purification was obtained. The molecular weight of the partially purified amylase enzyme from *B. megaterium* ATCC was 20 kDa. This study also proved that amylase enzyme from *B. megaterium* ATCC was successfully remove the biofilm of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* This result also confirmed with the light microscope and fluorescent microscope. In future new species of the *Bacillus* can be isolated for the removal of biofilm. The efficiency of biofilm removal by *B. megaterium* can be enhanced by genetic modification.

**DECLARATIONS**

Ethics approval: Not applicable
Consent to participate: Not applicable
Availability of data and material: Not applicable
Competing interest: Not applicable Funding: Not applicable
**Acknowledgement**: Not applicable

**Conflict of interest:** Authors declares no conflict of interest

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**Table 1: Statistical analysis of the medium components for the amylase production as per the Plackett – Burman design**

| Factor | Medium component | Effect Exi | S. E | t(xi) | P-value | Confidence level (%) |
|---|---|---|---|---|---|---|
| **X1** | **Starch** | 3290.5 | 1139512.25 | 1139512.25 | 0.010 | **98.91** |
| **X2** | **Peptone** | -769.25 | 1139512.25 | -0.06750 | 0.655 | 34.46 |
| **X3** | **MgSO4** | -7863.25 | 1139512.25 | -0.006900 | 0.0003 | **99.96** |
| **X4** | **CaCl2** | -4102.5 | 1139512.25 | -0.003600 | 0.0045 | **99.54** |
| **X5** | **NaCl** | -1324.75 | 1139512.25 | -0.0011625 | 0.2634 | 73.65 |

**Table 2: Analysis of variance of CCD for amylase production from *Bacillus megaterium***

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 8.514E+07 | 9 | 9.461E+06 | 6.28 | 0.0041 | **Significant** |
| A-Starch | 9.434E+06 | 1 | 9.434E+06 | 6.26 | 0.0313 | |
| B-MgSO4 | 6.914E+06 | 1 | 6.914E+06 | 4.59 | 0.0578 | |
| C-CaCl2 | 2.781E+06 | 1 | 2.781E+06 | 1.85 | 0.2040 | |
| AB | 6.311E+05 | 1 | 6.311E+05 | 0.4191 | 0.5320 | |
| AC | 1.197E+07 | 1 | 1.197E+07 | 7.95 | 0.0182 | |
| BC | 1.791E+06 | 1 | 1.791E+06 | 1.19 | 0.3010 | |
| $A^2$ | 1.654E+07 | 1 | 1.654E+07 | 10.98 | 0.0078 | |
| $B^2$ | 2.261E+07 | 1 | 2.261E+07 | 15.02 | 0.0031 | |
| $C^2$ | 2.261E+07 | 1 | 2.261E+07 | 15.02 | 0.0031 | |
| Residual | 1.506E+07 | 10 | 1.506E+06 | | | |
| Lack of Fit | 1.173E+07 | 5 | 2.346E+06 | 3.53 | 0.0965 | **not significant** |
| Pure Error | 3.327E+06 | 5 | 6.655E+05 | | | |
| Cor Total | 1.002E+08 | 19 | | | | |

**Table 3: Purification profile of amylase enzyme**

| Purification step | Total activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) | Purification fold |
|---|---|---|---|---|
| Crude extract | 8329.18 | 85.65 | 97.27 | 1 |
| Ammonium Sulphate Precipitation (60%) | 1871.94 | 15 | 124.7 | 1.28 |
| Dialyzed sample | 1782.11 | 12 | 148.5 | 1.53 |

**Figure 1. Primary screening of *Bacillus megaterium* ATCC**

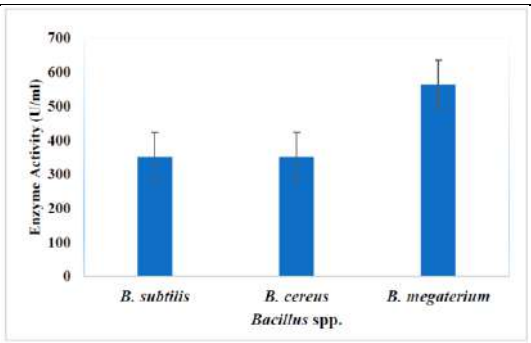


**Figure 2. Secondary Screening of *Bacillus* spp. for the production of amylase enzyme.**

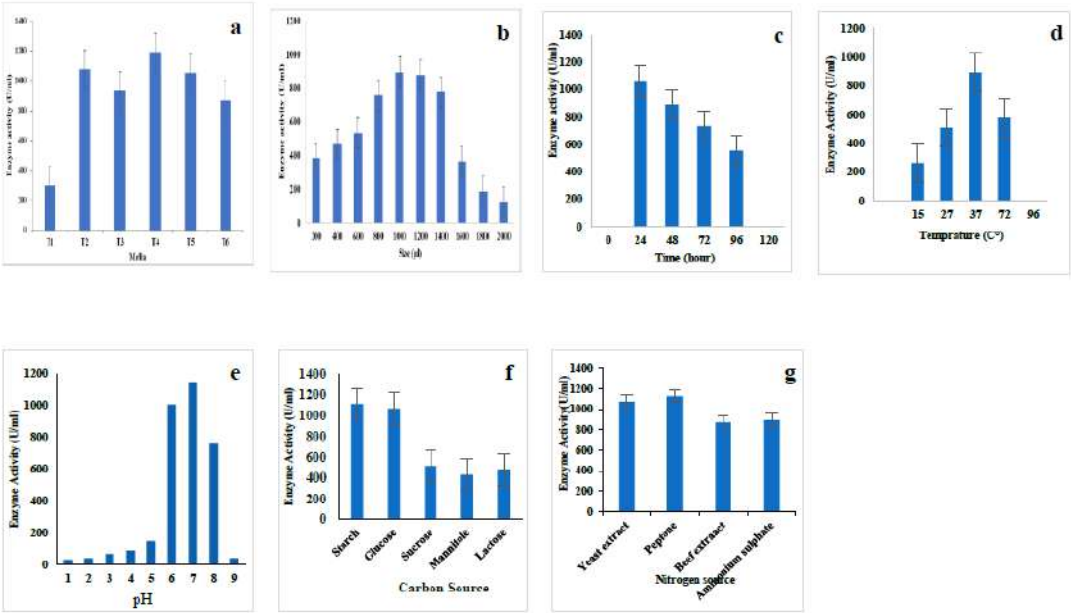


**Figure 3. Effect of (a) Moistening medium, (b) Inoculum size, (c) Time, (d) Temperature, (e) pH, (f) Carbon sources and (g) Nitrogen sources**

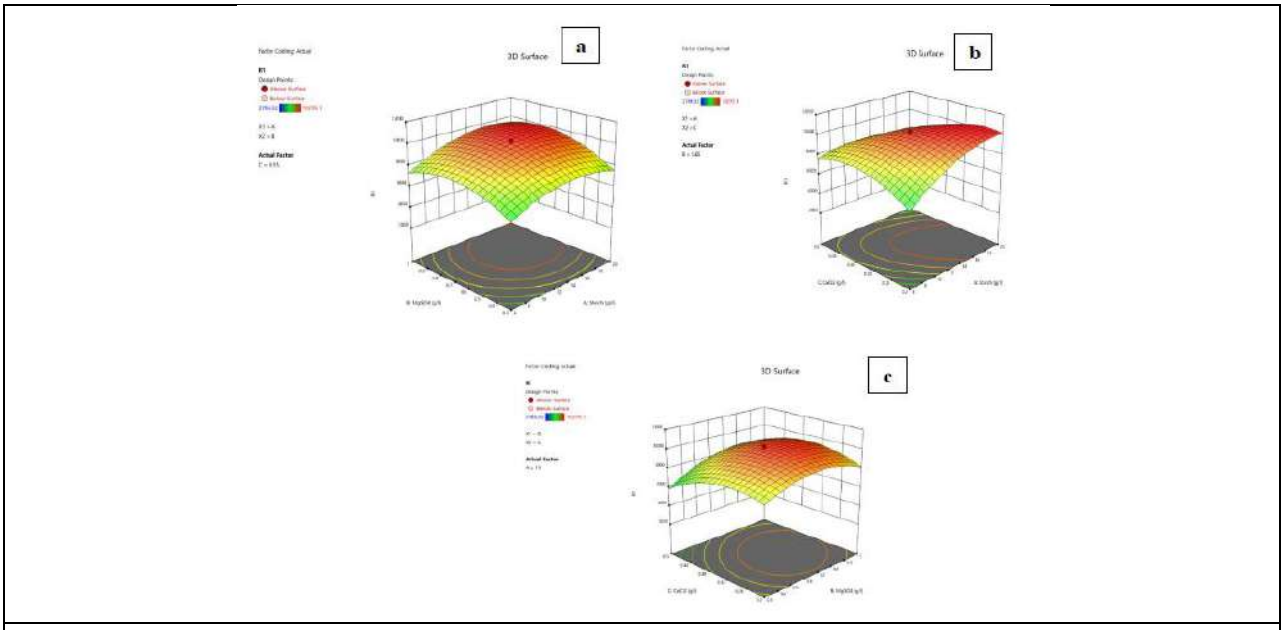


**Figure 4. 3 D Plot showing the (a) effect of $MgSO_4$ and starch, (b) CaCl2 and starch, (c) $CaCl_2$ and $MgSO_4$ on the Production of amylase enzyme.**

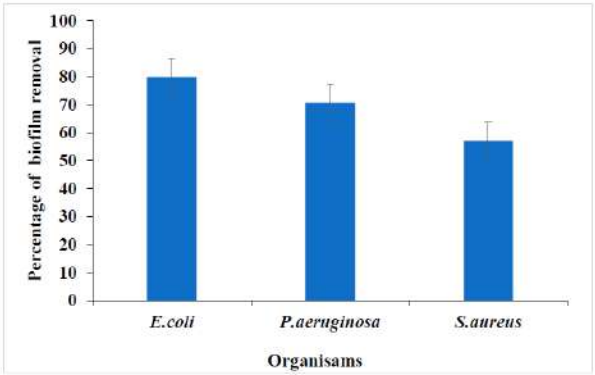


**Fig. 5 Biofilm removal by amylase enzyme**

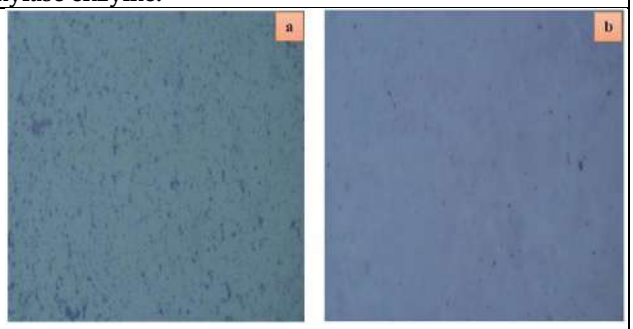


**Figure 6. (a) Positive Control of *E. coli,* (b) Biofilm treated by amylase**

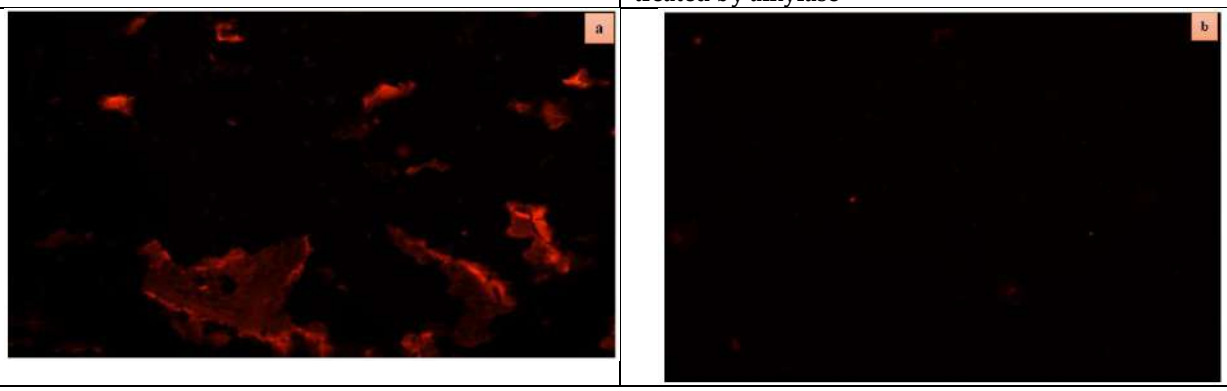


**Figure 7. (a) Fluorescence image of biofilm formation by *E. coli,* (b) Fluorescence image of treated biofilm by amylase enzyme.**

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**REVIEW ARTICLE**

# Linezolid - Induced Serotonin Syndrome in Clinical Practice: A Comprehensive Review

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## ABSTRACT

Serotonin syndrome (SS) represents a potentially life-threatening condition often overlooked due to its subtle initial symptoms. Linezolid, a commonly prescribed antibiotic for complex skin infections such as diabetic foot infections, can trigger SS. The syndrome primarily involves the neurotransmitter 5-Hydroxytryptamine (5-HT), commonly known as serotonin, which plays a central role in its pathophysiology. Serotonin synthesis occurs through the decarboxylation and hydroxylation of L-tryptophan. Recent research also highlights the involvement of dopaminergic and noradrenergic systems in SS.Diagnosis typically follows changes in medication or instances of self-poisoning and relies on the Hunter Serotonin Toxicity Criteria (HSTC). These criteria focus on signs of autonomic hyperactivity, mental status changes, and neuromuscular abnormalities. Various drugs and the discontinuation of specific medications can contribute to SS. Among these, linezolid poses a notable risk due to its weak inhibition of monoamine oxidase (MAO). Despite limitations, case reports provide valuable insights into the relationship between linezolid and SS. Prevention requires a comprehensive approach, with prompt drug discontinuation playing a critical role in management. Healthcare providers must recognize the contraindication of linezolid with other MAO inhibitors and remain vigilant for early signs of SS. Management strategies vary, with some cases resolving without intervention. This review highlights the

association between linezolid and SS, emphasizing the importance of further research to improve understanding and management of this life-threatening condition.

**Keywords**: Serotonin syndrome, linezolid, 5-Hydroxytryptamine, Hunter Serotonin Toxicity Criteria, monoamine oxidase.

## INTRODUCTION

Serotonin syndrome (SS) is a potentially fatal condition that clinicians often overlook due to its subtle early symptoms. This syndrome is not limited to psychotropic drugs; various substances, including fentanyl, meperidine, ondansetron, ritonavir, sumatriptan, dextromethorphan, and certain herbal products, can also trigger it [1]. Clinicians frequently fail to recognize the wide range of potential triggers, leading to misdiagnosis and underestimation of its severity. Increasing awareness among healthcare professionals about these diverse triggers is essential for early detection and effective management of SS [1]. Linezolid, a commonly prescribed antibiotic for complex skin infections such as diabetic foot infections, is a notable trigger of SS. The risk significantly increases when linezolid, a mild monoamine oxidase inhibitor, is combined with other serotonergic medications. This combination enhances serotonin release, heightening the likelihood of SS [2]. Raising awareness of these risks is critical for improving patient outcomes and preventing severe complications.

**Understanding Serotonin Syndrome: Historical Context and Modern Insights**

Serotonin syndrome (SS) is a potentially life-threatening adverse drug reaction that occurs due to inadvertent drug interactions, therapeutic drug use, or intentional self-poisoning [3]. This syndrome results from a predictable excess of serotonergic agonism in the central nervous system (CNS) and peripheral serotonergic receptors, distinguishing it from idiopathic drug reactions [4]. Serotonin toxicity, also known as serotonin syndrome, involves the overactivation of both central postsynaptic and peripheral 5HT-1A and 5HT-2A receptors [5]. Historical and contemporary insights reveal that episodes of "convulsive ergotism," reported between 1085 and 1927 in regions east of the Rhine River, were caused by ergot alkaloids in grains. These alkaloids are now recognized as precursors to serotonin syndrome, suggesting that this condition existed long before its formal identification in modern pharmacology [6]. This historical context highlights the gradual evolution in the understanding of SS.

The neurotransmitter 5-Hydroxytryptamine (5-HT), first identified in 1948, has been central to advancing knowledge about serotonin syndrome [7]. The term "serotonin syndrome" was first introduced in 1980 to describe specific symptoms observed in rats, marking a critical milestone in the clinical recognition of the syndrome [8]. This progression underscores the significance of both historical and modern research in shaping current perspectives on SS.

**Synthesis of Serotonin:**

Serotonin synthesizes through the decarboxylation and hydroxylation of L-tryptophan. This process is regulated by metabolizing enzymes, feedback loops, and reuptake mechanisms, ensuring balanced serotonin levels in the body. In serotonin syndrome (SS), several serotonin receptors are involved, with the 5-hydroxytryptamine (5-HT) receptors, particularly the 5-HT2A subtype, playing a central role [1]. The 5-HT1A receptor also contributes significantly by saturating receptor subtypes, leading to increased synaptic concentrations of serotonin agonists [9, 10]. However, the complexity of SS extends beyond serotonin receptor activity. Emerging research indicates that dopaminergic receptors and noradrenergic hyperactivity in the central nervous system may also influence serotonin production, though their exact roles remain unclear [11]. This underscores the need for further research to better understand the intricate mechanisms of serotonin regulation and its associated disorders.

**Diagnosis of Serotonin Syndrome**

Serotonin syndrome (SS) typically presents rapidly following a change in medicine or self-poisoning. In about 60% of cases, symptoms manifest within six hours of initiating a new drug, adjusting dosage, or overdosing, highlighting the syndrome's rapid onset [12]. Crucial for diagnosis are key neuromuscular symptoms, which include various forms of clonus, myoclonus, hyperreflexia, incoordination, peripheral hypertonicity, tremor, and shivering. These symptoms are central to identifying the syndrome [9, 10, 13-19]. Additionally, other clinical signs such as tachycardia, mydriasis, diaphoresis, hyperactive bowel sounds, diarrhea, agitation, and delirium are associated with serotonin syndrome, further complicating its clinical presentation [9, 12-16].

The diagnosis of serotonin syndrome (SS) relies on three primary classification systems: the Sternbach Criteria (SC), the Radomski Criteria (RC), and the Hunter Serotonin Toxicity Criteria (HSTC). Each system aims to identify characteristic symptoms and patterns associated with serotonin toxicity [13]. Among these, the HSTC is the most widely used. It focuses on classic features such as generalized clonus (inducible, ocular, and spontaneous), diaphoresis, agitation, tremor, and hyperreflexia. These criteria highlight the importance of specific symptoms in diagnosing the condition [13]. Spontaneous clonus, which involves rhythmic large muscle contractions, differs from myoclonic jerks and is typically triggered by minor movements, adding complexity to the diagnostic process [18]. Ocular clonus, characterized by involuntary, oscillatory eye movements, may occur continuously or be induced by eye movement. Additionally, rarer eye movements, such as the regular alternating gaze deviation known as "ping-pong" gaze, also contribute to the diagnostic framework for SS [18]. These features underscore the nuanced clinical presentation of SS and the importance of thorough assessment for accurate diagnosis.

**Recognizing Signs, Symptoms, and Severity of Serotonin Syndrome**

Serotonin syndrome (SS) typically manifests as a clinical triad of mental-status changes, neuromuscular abnormalities, and autonomic hyperactivity, although not all these features are consistently present in every patient (20). The severity of SS varies, ranging from mild to life-threatening forms. In mild cases, patients may experience tremors or muscle twitching, anxiety, hyperreflexia, tachycardia, diaphoresis, and mydriasis, often associated with recent use of antidepressants or over-the-counter medications containing dextromethorphan (21). Moderate toxicity causes significant distress, with symptoms such as restlessness, agitation, clonus in the lower extremities, hyperreflexia, opsoclonus, diarrhea, hyperactive bowel sounds, nausea, vomiting, tachycardia, hypertension, hyperthermia (below 40°C or 104°F), and mydriasis. This presentation frequently follows the use of substances like ecstasy or combinations of serotonin-potentiating agents, including antidepressants, proserotonergic opioids, antiepileptics, or CYP2D6 and CYP3A4 inhibitors (22). Severe toxicity, a life-threatening condition, can lead to multiorgan failure within hours. Muscle rigidity significantly elevates body temperature above 40°C, potentially masking typical signs such as clonus and hyperreflexia. Patients often exhibit unstable delirium, confusion, tonic-clonic seizures, and erratic vital signs. Without timely intervention to manage muscle rigidity and hyperthermia, complications like myoglobinuria, rhabdomyolysis, metabolic acidosis, renal failure, disseminated intravascular coagulation, and acute respiratory distress syndrome may develop, further increasing the risk of mortality (23).

**Drug Related Causes of Serotonin Syndrome**

A wide range of drugs and drug combinations is associated with serotonin syndrome (SS), spanning several categories. These include tricyclic antidepressants, monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), and opioid analgesics. Over-the-counter cough medications, certain antibiotics, weight-loss agents, antiemetic drugs, antimigraine agents, substances of abuse, and herbal products are also implicated. Notably, the onset of SS can result not only from the use of specific medications but also from their cessation [24-26].

Drugs that inhibit serotonin breakdown represent a significant category of SS triggers. This group includes MAOIs, linezolid [27], procarbazine, methylene blue, and Syrian Rue. Another critical category consists of drugs that block serotonin reuptake, such as serotonin-norepinephrine reuptake inhibitors (SNRIs), SSRIs, tricyclic antidepressants, and opioids like meperidine, tramadol, buprenorphine, dextromethorphan, and tapentadol. Certain antiepileptic drugs, including valproate and carbamazepine, and antiemetic medications such as granisetron, ondansetron, and metoclopramide, along with the herbal supplement St. John's Wort, also belong to this category.

Drugs that increase serotonin precursors or act as agonists further contribute to SS. Examples include lithium, tryptophan, fentanyl, and lysergic acid diethylamide (LSD). Similarly, agents that enhance serotonin release, such as amphetamines, fenfluramine, and methylenedioxymethamphetamine (ecstasy), play a role in the syndrome's development. Additionally, inhibitors of CYP2D6 and CYP3A4 enzymes, including erythromycin [25], ciprofloxacin, ritonavir, fluconazole, and grapefruit juice, can prevent the breakdown of serotonergic agents, exacerbating the risk of SS.However, it is important to note that only MAOIs, SSRIs, SNRIs, and serotonin releasers are reliably confirmed as direct causes of SS. Other drug interactions, although documented in case reports, require further comprehensive evaluation to establish their role in SS [28, 29].

**Linezolid and its clinical implications**

Oxazolidinones, first discovered and developed in the 1970s, were originally intended for treating plant diseases [30]. This class of compounds achieved a significant milestone with the development of linezolid. Approved by the Food and Drug Administration (FDA) in 2000, linezolid became the first oxazolidinone antibiotic authorized for human use, marking a pivotal advancement in antibiotic therapy [31].The FDA approval of linezolid includes indications for treating complicated and uncomplicated skin and skin-structure infections, nosocomial pneumonia, and community-acquired pneumonia. It is also effective against infections caused by methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) [32].

Registry trials closely examine linezolid's safety profile. The most commonly reported adverse effects include nausea, headache, diarrhea, vomiting, and myelosuppression, such as thrombocytopenia, anemia, and leukopenia, which are associated with prolonged use. However, serious adverse effects are rare and do not significantly differ from those observed in comparison groups [33].Linezolid acts by binding to the bacterial 23S ribosomal RNA of the 50S subunit, preventing the formation of a functional 70S initiation complex [34]. This mechanism inhibits the initiation of protein synthesis, reducing the length of peptide chains and slowing the translation process. As a synthetic antibiotic, linezolid specifically blocks bacterial protein synthesis [35]. Its mechanism of action occurs at the initiation stage, unlike other protein synthesis inhibitors that target the elongation phase. Due to this unique site of action, cross-resistance with other protein synthesis inhibitors has not been observed [36].

**Potential Risk of Serotonin Syndrome with Linezolid**

Linezolid, an oxazolidinone antimicrobial approved by the FDA in 2000, is widely used to treat complex infections caused by drug-resistant gram-positive cocci, including vancomycin-resistant Enterococcus and methicillin-resistant Staphylococcus aureus. Initial studies reported no cases of serotonin toxicity; however, post-marketing reports document serotonin toxicity when linezolid is combined with serotonergic agents [37]. Before FDA approval, research identified linezolid as a weak competitive (reversible) inhibitor of monoamine oxidase (MAO), an enzyme that deactivates neurotransmitters like serotonin, epinephrine, norepinephrine, and dopamine [38]. This raised concerns about the potential for serotonin syndrome when linezolid is used with serotonergic agents. Despite these concerns, clinical trials did not observe clinical evidence of MAO A inhibition [39]. Post-approval reports, however, highlight emerging cases of serotonin toxicity [40].

In response, the FDA issued a warning in 2011 about the potential for serotonin toxicity when linezolid is co-administered with serotonergic psychiatric medications. This warning is based on case reports and data from the FDA Adverse Event Reporting System (AERS) [41]. Lawrence et al. [42] analyzed post-marketing reports from AERS and identified 29 cases of linezolid-associated serotonin toxicity, with three meeting the Hunter Serotonin Toxicity Criteria (HSTC). Most cases involved selective serotonin reuptake inhibitors (SSRIs), affecting patients aged 17 to 83 years. This finding aligns with a review by Boyer and Shannon [1], which highlighted a potential drug interaction between ritonavir and linezolid, although no definitive case reports have directly linked these two agents to serotonin syndrome.

The variability in AERS reports is significant, with consumer submissions accounting for nearly half of all reports in 2010, totaling 403,746 cases [43]. Given that both consumers and healthcare providers contribute to the AERS, reports

may be incomplete or inaccurate, underscoring the need for careful evaluation. Reflecting these concerns, the FDA issued a MedWatch Safety Alert in July 2011, warning of severe central nervous system reactions when linezolid is combined with serotonergic psychiatric medications. As a result, safety warnings about serotonin toxicity were added to the labels of linezolid and serotonergic drugs [37]. In October 2011, the FDA further advised against using linezolid in patients taking serotonergic medications unless the benefit outweighs the risk. Clinical research supports these regulatory actions. Woytowish and Maynor [44] identified 32 cases of linezolid-associated serotonin toxicity, including three fatalities, in a literature review. Most cases involved SSRIs, with symptom onset and resolution varying from hours to days. Despite these reports, they concluded that serotonin toxicity with linezolid is rare. A 2012 retrospective analysis of Phase 3 and 4 clinical trials involving approximately 4,000 patients found no serotonin toxicity cases among those using linezolid or comparators with serotonergic agents [38]. This suggests that while a risk exists, the incidence is low.

Further studies corroborate these findings. Go et al. [45] conducted a retrospective analysis at the University of Colorado Hospital, examining serotonin toxicity in hospitalized patients receiving linezolid with escitalopram or citalopram. They reported only one mild case, suggesting severe reactions are uncommon. Similarly, Lorenz et al. [46] reviewed serotonin toxicity in patients on linezolid with SSRIs or serotonin-norepinephrine reuptake inhibitors (SNRIs). Only 1.9% of patients met HSTC criteria, reinforcing the rarity of severe cases. Taylor et al. [47] also reviewed cases of patients on linezolid and serotonergic agents, finding that only 2.8% had a high probability of serotonin toxicity. Despite these findings, caution remains critical. Lodise et al. [48] argue that serotonin toxicity should not prevent linezolid use in patients taking serotonergic drugs, as no increased risk is observed compared to vancomycin. Similarly, Packer and Berman [49] report that combining linezolid with antidepressants is not contraindicated, although antidepressants may be withdrawn in certain cases. However, the FDA continues to receive reports of serotonin syndrome linked to the combined use of linezolid and serotonergic medications, including fatal cases [50]. Based on this evidence, the FDA concludes that this drug interaction is likely, particularly when long-half-life serotonergic medications are discontinued. Therefore, linezolid should generally not be prescribed to patients on serotonergic drugs unless the benefits outweigh the risks [51].

**Incidence of Serotonin Toxicity in Patients Treated with Linezolid: Real life cases and research**

Case reports provide critical details such as patient gender, age, complete medication regimens, time to diagnosis, and resolution of serotonin toxicity. Retrospective and prospective studies further contribute data, focusing on methodology, participant numbers, diagnostic criteria for serotonin toxicity, associated serotonergic agents, and the duration of linezolid therapy [40]. Randomized controlled trials (RCTs) report no cases of linezolid-induced serotonin toxicity, but smaller retrospective and observational studies document its occurrence. The incidence of serotonin toxicity among patients treated with linezolid alone is 0.005%, compared to 0.0134% in those receiving linezolid in combination with other therapies. While this suggests a modest increase in risk with combination therapy, the overall incidence remains low [40]. A single-center, retrospective cohort study conducted between January 1, 2014, and June 30, 2021, examines hospitalized patients aged 18 years and older who received at least one dose of linezolid, with or without serotonergic agents [59].

Previous retrospective cohort studies report varying incidences of serotonin toxicity, ranging from 0.54% to 18.2%, in patients receiving linezolid alongside serotonin reuptake inhibitors (SRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs) [38, 44, 46, 47, 60]. Given the variability in these findings and limitations in sample sizes, contemporary data is necessary to confirm the rarity of serotonin toxicity. A recent study involving 1,743 patients treated with linezolid, with or without serotonergic agents, provides important insights. Among this cohort, 67% (1,168/1,743) were on more than one serotonergic agent, with 52.7% (616/1,168) receiving moderate to high doses. Despite this potentially high-risk population, the incidence of serotonin toxicity remains low: 0.06% (1/1,743) based on the Sternbach Criteria and 0% (0/1,743) based on the Hunter Criteria, which is more accurate for diagnosis [13, 61]. In cases like Patient B, where serotonin toxicity is unlikely, linezolid's role is minimal, as symptoms appeared 16 days after discontinuation [59].

Analysis of the FDA Adverse Event Reporting System (AERS) highlights the prevalence of serotonin toxicity when linezolid is combined with serotonergic agents [42]. Notably, 69% of published cases involve selective serotonin reuptake inhibitors (SSRIs), with citalopram being the most frequently implicated agent in eight cases. Other commonly associated agents include sertraline and venlafaxine, each linked to seven cases. These reports involve linezolid either alone or in combination with additional serotonergic agents. Fluoxetine is implicated in three cases, one of which occurred despite discontinuation 18 days before linezolid administration. Meperidine is associated with three cases of serotonin toxicity, while paroxetine, escitalopram, tramadol, and duloxetine are each linked to two cases. Half of the 32 patients in these reports received more than one serotonergic agent, highlighting the complexity of drug interactions in serotonin toxicity [45-47, 50, 62-69].

**Prevention and safety measures**

Preventing serotonin syndrome requires a multifaceted approach that includes pharmacogenomic research, physician education, changes in prescribing practices, and technological advancements. Pharmacogenomic principles may help identify patients at risk for serotonin syndrome before administering serotonergic agents [52]. When toxicity occurs, consulting a clinical pharmacology service, medical toxicologist, or poison-control center can help identify proserotonergic agents and drug interactions. These resources provide valuable insights into anticipating adverse effects and assist clinicians in making informed decisions [52]. Avoiding multidrug regimens is critical in preventing serotonin syndrome. When multiple agents are necessary, tools such as personal digital assistants and computer-based ordering systems can detect drug interactions and reduce reliance on memory during prescribing. Additionally, post-marketing surveillance and physician education increase awareness of serotonin syndrome [52].

When serotonin syndrome occurs, prompt discontinuation of the causative drugs is essential for management. Administering 5-hydroxytryptamine antagonists, such as cyproheptadine, is recommended, with therapeutic doses for adults ranging from 12–32 mg over 24 hours, binding 85%–95% of serotonin receptors [53]. Benzodiazepines can be used to manage agitation, while supportive care remains central to controlling autonomic instability and hyperthermia [53]. This approach emphasizes the importance of immediate and targeted interventions in managing serotonin syndrome.

To reduce the risk of serotonin syndrome during medication transitions, adhering to appropriate washout periods is critical [54, 55]. These periods vary based on the half-life of medications and are necessary to prevent overlapping effects of serotonergic agents. For example, sertraline requires a two-week washout period, while fluoxetine requires 5 to 6 weeks [56]. Consulting pharmacists can help navigate these complexities, highlighting the value of interdisciplinary collaboration in medication management. Patient education on dietary restrictions is also essential, particularly when prescribing medications such as linezolid that interact with dietary tyramine. Patients should avoid tyramine-rich foods and beverages, as consuming large quantities can cause severe hypertensive reactions in individuals taking strong MAO inhibitors [57]. Examples of these foods include aged cheeses, cured meats, and certain liquors such as vermouth. Patients should also limit red and white wine consumption to no more than 120 ml per meal. This guidance is based on findings that systolic blood pressure rises significantly when linezolid is coadministered with 100 mg or more of tyramine [58]. These precautions emphasize the importance of dietary considerations in ensuring medication safety.

**Guidelines for Healthcare Providers**

Linezolid, a reversible monoamine oxidase inhibitor (MAOI), is contraindicated with other MAOIs such as phenelzine, moclobemide, and selegiline, or if these agents have been used within the preceding two weeks [70]. The Summary of Product Characteristics (SPC) recommends caution when prescribing linezolid to patients with certain clinical conditions or those on specific medications. These conditions include uncontrolled hypertension, carcinoid, thyrotoxicosis, phaeochromocytoma, schizoaffective disorder, bipolar depression, and acute confusional states. Patients taking medications such as serotonin reuptake inhibitors, tricyclic antidepressants, serotonin 5-HT1 receptor agonists (triptans), sympathomimetic agents (e.g., pseudoephedrine, adrenergic bronchodilators, phenylpropanolamine), vasopressive agents (e.g., norepinephrine, epinephrine), dopaminergic agents (e.g., dopamine, dobutamine), opioid analgesics (e.g., pethidine, methadone, tramadol, dextromethorphan), or buspirone

should be closely monitored when linezolid is prescribed [70]. Healthcare providers must watch for signs of serotonin syndrome, including hypertension, tachycardia, cognitive dysfunction, hyperpyrexia, hyperreflexia, and incoordination. Rifampicin, which reduces linezolid concentrations by 30% due to the induction of the p-glycoprotein transporter, should be avoided. Rifampicin co-administration is not recommended, and it should be discontinued at least two weeks before initiating linezolid therapy [70].

**Communicating the Risks**

Patients should be informed about potential side effects of linezolid, such as dizziness and visual impairments, and advised not to drive or operate machinery if these occur. Any visual disturbances should be immediately reported to their healthcare provider. Patients should also exercise caution with their diet. Foods rich in tyramine, such as aged cheese, yeast extracts, soybean extracts, draft beers, and wine, should be minimized. Additionally, over-the-counter medications, including decongestant cold and flu remedies, should only be taken after consulting a healthcare provider to prevent unintended interactions [70].

**Educating Patients**

Patients should be made aware that serotonin syndrome is a potentially life-threatening condition that can occur with linezolid. Symptoms include agitation, confusion, rapid heartbeat, high blood pressure, muscle rigidity, sweating, fever, diarrhea, and hallucinations. Patients must seek immediate medical attention if they experience any of these symptoms. The risk increases when linezolid is combined with serotonergic medications, such as selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), or triptans for migraines. Patients should disclose all medications, including supplements and over-the-counter drugs, to their healthcare provider to avoid harmful interactions [51]. Patients should not adjust their medication regimen, including dosages, without consulting their healthcare provider. Promptly reporting symptoms of serotonin syndrome improves prognosis. Healthcare providers should monitor patients closely when linezolid is combined with serotonergic drugs. If necessary, alternative treatments should be considered, and patients should discuss these options with their provider [51]. Effective risk management requires clear communication and strict adherence to prescribed medication regimens [51].

**Management of Serotonin Syndrome: Case Studies and Therapeutic Approaches**

In many cases, serotonin syndrome resolves with simple interventions, such as discontinuation of the causative drug or adjustments to antibiotic therapy. However, some cases require specific therapeutic interventions, including the management of hypertension, cholinergic symptoms, and fever, alongside addressing underlying psychiatric conditions. Such treatments can increase healthcare costs and prolong hospital stays. In four cases, serotonin syndrome caused hypertension requiring treatment [63, 71, 72]. One case involved a woman with a blood pressure of 150/90 mm Hg, who required monitoring [71]. Other patients, including a 37-year-old man and a 56-year-old woman, were treated with oral β-blockers such as bisoprolol and propranolol [63, 71]. In one instance, the details of the antihypertensive medication used remain unspecified [72]. Two patients with severe hypertension required a combination of three antihypertensive therapies [63]. One of these patients, a 56-year-old woman, also received cyproheptadine to manage serotonin syndrome-related tremors and weakness.

Other treatments included anticholinergic and psychotropic medications. For example, a pediatric patient received diphenhydramine after discontinuing fluoxetine to alleviate clonic movements, agitation, and mydriasis [73]. Managing anxiety was another critical aspect. A male patient experienced severe anxiety after stopping mirtazapine and benefited from short-term benzodiazepine therapy [74]. Another male patient was readmitted due to hypertension, panic attacks, and anxiety. In his case, linezolid was discontinued a day early, and his olanzapine dosage was increased, along with the addition of methotrimeprazine, a phenothiazine antipsychotic [71].

**Significance of the review**

This review article provides essential insights for healthcare professionals by addressing Serotonin Syndrome (SS), a critical yet frequently overlooked medical condition. It focuses specifically on drug-induced SS, with an emphasis on

its association with the antibiotic linezolid. The review also offers practical guidelines for the safe use of linezolid, ensuring that healthcare providers can mitigate risks effectively. Additionally, it incorporates case studies that serve as valuable educational resources.

## CONCLUSION

This review highlights the link between linezolid use and serotonin syndrome (SS), emphasizing its risks when combined with serotonergic drugs. It underscores the need for ongoing research into linezolid's mechanisms, genetic predispositions, and prevention strategies. Recommendations include enhancing healthcare provider education, improving diagnostic tools, fostering interdisciplinary collaboration, and increasing patient awareness. Advancing pharmacovigilance systems and reporting adverse drug reactions are also crucial for reducing SS risk and improving patient safety and outcomes.

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**Table 1. Incidence of Serotonin Toxicity Connected to Use of Linezolid Plus Other Serotonergic Agents (38, 45-47)**

| Reference | Linezolid/ Serotonergic Agent, N | Serotonin Toxicity, n | Incidence,% |
|---|---|---|---|
| Butterfield (2012) | 2208 | 12[a] | 0.54 |
| Go (2010) | 24 | 1[b] | 4.17 |
| Lorenz (2008) | 53 | 1[b] | 1.89 |
| Taylor (2006) | 72 | 2[a] | 2.78 |

a. Serotonin toxicity diagnosed via Hunter Serotonin Toxicity Criteria or Sternbach's criteria.
b. Serotonin toxicity diagnosed via Hunter Serotonin Toxicity Criteria.

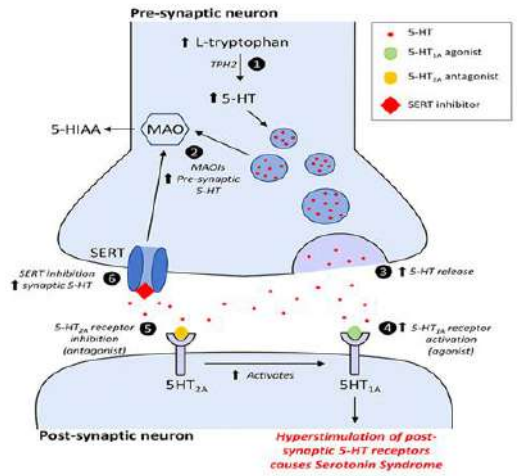


**Figure 1. illustrates the various pathways contributing to serotonin syndrome [11]. 1) an abundance of L-tryptophan elevates the production of 5-HT (serotonin), a reaction facilitated by tryptophan hydroxylase 2 (TPH2). 2) monoamine oxidase inhibitors (MAOIs) prevent the breakdown of serotonin, resulting in higher presynaptic levels. 3) substances like amphetamines, cocaine, MDMA (3,4-methylenedioxy-methamphetamine), and levodopa prompt the release of more 5-HT. 4) there's either direct or indirect stimulation of 5-HT1A receptors on the postsynaptic side. 5) the antagonism of 5-HT2A receptors may amplify the effects of 5-HT1A receptor agonists. 6) the inhibition of the serotonin reuptake transporter protein (SERT) by selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) leads to increased serotonin levels in the synaptic gap.**

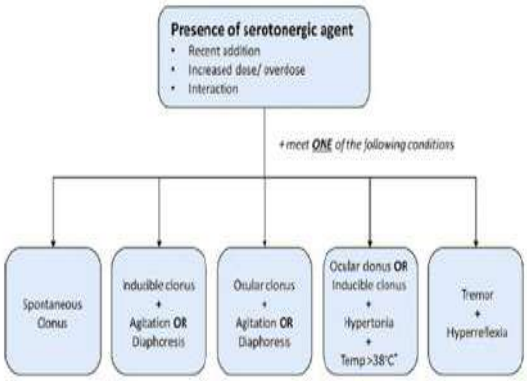


**Figure 2. The Hunter Criteria for Serotonin Toxicity are essential for identifying serotonin syndrome [11]. Central to this diagnosis is the necessity for some type of neuromuscular excitement. The presence of a temperature of 38.5°C or higher, along with pronounced hypertonia or rigidity, particularly in the trunk, signals a severe form of serotonin syndrome. This condition poses a risk of worsening, potentially leading to respiratory difficulties.**

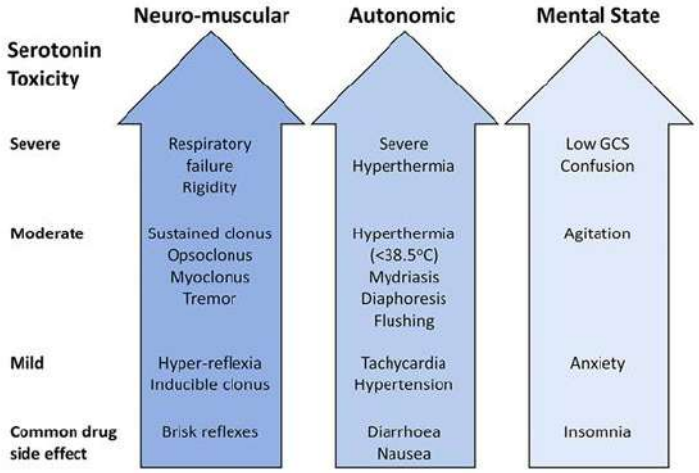


**Figure 3. The scope of clinical features in SS [18]. GCS= Glasgow Coma Scale.**

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**RESEARCH ARTICLE**

# Enhancement in Solubility and Dissolution Rate of Glimepiride using Spherical Crystallization Technique

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## ABSTRACT

Improving the solubility and dissolution rate of drugs with poor water solubility remains a significant hurdle in the pharmaceutical industry. Glimepiride, an oral medication used for managing diabetes, is a notable example of a compound with low aqueous solubility that necessitates enhancement strategies. This research focuses on utilizing spherical crystallisation to increase the solubility and dissolution rate of glimepiride. The method integrates crystallization and aggregation into a single-step process, resulting in spherical agglomerates with superior physicochemical attributes. Spherical crystals of glimepiride were produced using the solvent-change approach, involving the use of an appropriate combination of good solvent, poor solvent, and bridging liquid. The resulting agglomerates were analyzed for their particle size, morphology, flow characteristics, and mechanical properties using tools such as scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). Solubility and dissolution studies were conducted following standard procedures, with findings compared to unprocessed glimepiride.The spherical agglomerates showed remarkable improvements in solubility and dissolution rates compared to the untreated drug, which could be attributed to enhanced surface area, reduced crystallinity, and better wettability. Furthermore, the agglomerates demonstrated superior flow properties, making them ideal for direct tablet compression. This investigation underscores the potential of spherical crystallization in overcoming the limitations of poorly water-soluble drugs like glimepiride, offering a viable solution to enhance therapeutic efficacy and patient adherence.

**Keywords:** Glimepiride, spherical crystallization, solubility enhancement, dissolution rate, poorly water-soluble drugs, solvent-change method, particle engineering, crystallinity reduction.

## INTRODUCTION

Diabetes is a metabolic disorder marked by high blood sugar levels due to insulin production issues or insulin resistance. Managing diabetes often involves medications, especially for type 2 diabetes, where oral drugs are commonly prescribed. A significant challenge with many of these drugs is poor solubility, which can reduce their effectiveness. Cocrystallization, a process that involves combining a drug with conformers to form a crystalline structure, offers a solution by improving the solubility and bioavailability of poorly soluble drugs. Cocrystallization enhances the solubility and dissolution rate of drugs, making them more effective when taken orally. This is particularly important for diabetes medications like metformin, which is essential for managing type 2 diabetes but suffers from poor water solubility. Cocrystallization increases the drug's dissolution rate, improving absorption in the gastrointestinal tract and ensuring better therapeutic outcomes. The process involves combining the drug molecule with one or more coformers to form a cocrystal. These cocrystals retain the drug's therapeutic properties but improve its solubility, making it easier for the body to absorb. This approach allows for more consistent blood sugar control, potentially reducing the side effects often linked to poorly soluble drugs. Metformin, the first-line treatment for type 2 diabetes, has low solubility, which can lead to inconsistent absorption and side effects like gastrointestinal discomfort. Research into metformin cocrystals has shown promising results in enhancing its solubility, reducing side effects, and improving its absorption. By modifying metformin's crystalline form, the drug can achieve more reliable blood glucose control.[1,2].

Other diabetes drugs, including rosiglitazone and glibenclamide, have also been studied for co-crystallization. These medications, like metformin, can benefit from improved solubility, which may lead to more consistent therapeutic effects and fewer adverse reactions. Cocrystals improve the solubility of poorly water-soluble drugs, allowing for better absorption and more effective treatment. Enhanced solubility can lead to lower required doses, which may help reduce side effects such as nausea and gastrointestinal discomfort. Cocrystals can be designed to release the drug gradually, providing steady blood sugar control and reducing the need for frequent dosing. Regulatory bodies like the FDA have recognized cocrystallization as a viable approach to improving drug formulations, making it an appealing method for drug development. While cocrystallization offers many advantages, challenges remain. Identifying suitable coformers and optimizing formation conditions require careful research. Additionally, stability and shelf-life considerations for cocrystals must be addressed to ensure their long-term effectiveness. Nevertheless, cocrystallization represents a promising strategy for improving diabetes drug formulations. By enhancing solubility and bioavailability, cocrystals could lead to better absorption, fewer side effects, and more controlled drug release, offering improved blood sugar management for diabetes patients. In conclusion, cocrystallization holds significant potential for revolutionizing diabetes treatment. By overcoming solubility challenges, it can provide more effective medications, improving patient outcomes and enhancing the management of diabetes.[3,4]

## MATERIAL AND METHOD

**Preparation of spherical agglomerates of glimepiride by spherical crystallization technique:**All reagents used in this investigation were of analytical grade and double distilled water was used. The Glimepiride (1gm) was dissolved in dimethyl formamide, dichloromethane and methanol (30 ml) in a small beaker. Distilled water (100 ml) was added to precipitate the drug in fine crystals. The mixture was allowed to stand for 10 minutes. It was then stirred using three blade electric stirrers at 600 rpm and chloroform (5 ml) was added drop wise as a bridging liquid while stirring. This system was agitated for 45 minutes at room temperature. Spherical crystals were separated by filtration and dried in oven at 400C73-74 for 20 min.[5,6]

**Evaluation**

The formation of co crystals are identified by using different methods

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were obtained using a Bruker Alpha System with Spectrum opus 6.5 software. A KBr pellet of drug, carrier, adsorbent and the solid dispersion granules was prepared. The sample was dispersed in the KBr and ground using a mortar and pestle. The KBr pellet was prepared by the application of high pressure.

### Differential Scanning Calorimetry

DSC studies were performed using a DSC (Siio-6300, Japan instruments). It is operated using Stare Software V8.10. A 3-5 mg sample was weighed and placed into a 100 μL aluminium pan which is further crimped with a lid. The pan was then placed into the DSC unit along with a similar pan as a reference. The sample was scanned at a heating rate of 100C/min from 0 ºC to 300 ºC and purged under nitrogen gas flow rate of 50 ml/min. The DSC was calibrated using indium (5-10 mg) with a melting onset of 156 ± 0.2 ºC as the standard

### X-Ray powder Diffraction (XRPD)

XRPD was performed to observe the physical state of the spherical agglomerates and to evaluate any interaction between the drug, and solvents in spherical crystallization technique.. Philips PAN analytical X-Pert Pro V1.6 with X-Pert Data Collector V2.1 software was used equipped with a CuK$\alpha$2 anode tube and diffractometer of radius 240mm. The XRD scan was performed using BB004 flat stage. The powdered sample was placed in a plastic sample holder of 1 inch square. Data were collected at 45 kV and 40 mA. Samples were scanned from 0-40 º 2θ at a step size of 0.0084 and scan rate of 1.00 º/min[7,8]

### Saturation solubility studies

Solubility studies of the prepared spherical agglomerates of glimepiride (GD+DMF, GD+DCM, and GD+methanol spherical agglomerates) solutions of prepared agglomerates were placed in 50ml beakers and the prepared saturated solutions were kept in an orbital shaker for 72hrs at 25˚C with 300 rpm. After 72 hrs the samples sonicated for 2hours. Then Samples were filtered through 0.45 micro meter filters and their concentration was determined by using Elico SL 159 UV-spectrophotometer at 231 nm. The results were extrapolated to determine the percent w/v of Glimepiride in its saturated solution with the solvent under investigation.[9]

### *In- vitro* dissolution studies

The *in-vitro* release profiles of glimepiride spherical crystals by spherical crystallization technique were obtained using a dissolution test apparatus USP-II (Electro Lab). The dissolution study was carried out in 900 ml 0.1 N HCl as the dissolution medium at 37˚C ± 5˚C and 50rpm. Then 5 ml samples were collected for 5, 10,15, 20, 30, 45, 60, .The dissolution medium was replaced with 5 ml fresh dissolution fluid to maintain sink conditions. The withdrawn samples were filtered and analyzed, spectrophotometrically (Elico, SL 159) at 231 nm. The mean of two determinations was used to calculate the drug release from each of the formulations.

## RESULTS AND DISCUSSION

The study the compatibility of glimepiride with other solvents were prepared by mixing drug with solvents in the ratio of 1:1 and stored in air tight containers at room temperature and 30◦c /65%RH for three months .the solid admixtures were characterized using Fourier Transform infra-red spectroscopy. In FTIR studies Glimepiride showed characteristic peak at 3369(NH group (secondary amines ) ;3288(NH(amide);1707(C=O(carboxylic acid);1673(C=O(amide) in drug alone and in combination of pure drug with other solvents.

### DSC results and discussion

The compatibility of drug and solvent (DCM, DMF, methanol) were evaluated by DSC. the DSC thermogram of glimepiride exhibited a broad endothermic peak at 226.3◦c corresponding to its melting point 205 to 208◦C. the DSC thermo gram of G.D+DCM showed melting peak of drug at 250.2◦c , G.D +methanol agglomerates showed melting

peak at 263.8◦c and G.D+DMF showed melting peak of drug at 250.2◦c . This indicates no interaction between G.D and all the solvents in the spherical agglomerates.

**XRD results and discussions**

The physical state of a drug in the spherical agglomerates were evaluated by XRD the XRD of glimepiride exhibited a few characteristic diffraction peaks indicating its crystalline nature. In the XRD of G.D+DCM spherical agglomerates less no of diffraction peaks and their intensities were reduced indicating reduction in crystallinity .but in the XRD of G.D +methanol and G.D+DMF exhibited no of characteristic diffraction peak with high intensities indicating its more spherical crystallization.

**Determination of yield and drug content for prepared spherical agglomerates**

| Spherical agglomerates | Yield% | Drug content |
|---|---|---|
| G.D+DMF | 97±2 | 93±2 |
| G.D+DCM | 90±2 | 83±1 |
| G.D+methanol | 94±1 | 90±2 |

**Solubility Studies:** The solubility of glimepiride in distilled water,G.D+DMF,G.D+DCM and G.D+methanol was found to be 0.0619, 0.216, 0.0495, and0.194mg/ml respectively.

The solubility increases in the order as G.D +DMF>G.D+ methanol> G.D+DCM> pure drug.

The spherical crystallization of Glimepiride with DMF, DCM and methanol was investigated by saturation solubility studies in distilled water. The aqueous solubility of Glipizide was increased in spherical agglomerates made with DMF and methanol compared to pure Glipizide. The aqueous solubility of Glipizide in DMF and methanol spherical agglomerates increased 3.32 and 3.13times than that of the pure Glipizide respectively. But the aqueous solubility of Glipizide in DCM spherical crystals was similar to aqueous solubility of pure Glipizide. There is only 0.79 times that of the pure Glipizide. There is no much increase in solubility of drug in DCM spherical crystals.

***In vitro* release studies**

Glimepiride release from different spherical agglomerates was studied in 0.1N HCL for a period of 60 minutes . glimepiride release from all spherical agglomerates was rapid and released 100%. Dissolution efficiency (DE) is another parameter suitable for the evaluation of in vitro dissolution rate. DE is defined as the area under the dissolution curve up to a certain time (t) expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time .The dissolution efficiency can have a range of values depending on the time intervals should be chosen for comparison. For example, $DE_{15}$ would relate to the dissolution of the drug from a particular formulation after 15 minutes and could only be compared with $DE_{15}$ of other formulations .summation of the large dissolution data in to a single figure DE enables ready comparison to be made between a large numbers of spherical agglomerates .the second advantage is that it can be theoretically related to in vivo data. Since in vivo drug availability is estimated by integrating the area under blood level curve its seems reasonable to express in vitro results similarly. Also, when a relation is to be shown between dissolution and another variable, it is perhaps more realistic to use dissolution efficiency which takes in to account the dissolution profile as a whole, as opposed to $T_{50\%}$ or $T_{90\%}$ values which use just one point from the plot. Dissolution efficiency ($DE_{15}$) values were calculated as per khan. T50 (time taken for 50% dissolution) values were recorded from the dissolution profiles. The dissolution parameters are summarized in table

The formulations G.D +D.M.F, G.D + methanol got good dissolution efficiency values compared to G.D + D.C.M and pure (G.D) It indicates increase dissolution rate in G.D +D.M.F, G.D + methanol compared to G.D + D.C.M and pure (G.D)

## CONCLUSION

The overall objective of the present investigation is to enhance solubility Glimepiride, an anti-diabetic BCS class II drug and to enhance dissolution rate. Various methods used to enhance solubility were studied and reviewed literature. Among them spherical crystallization is one of the unique method to enhance solubility and also to enhance dissolution rate. Various solvents selected in the preparation of spherical agglomerates of the Glimepiride based on drug solubility and nature of the solvent. DMF, DCM and methanol selected as good solvents, chloroform as bridging liquid and distilled water as poor solvent. Spherical agglomeration technique was optimised by setting different RPMs and finally 600 rpm was selected. The drug and excipient compatibility study were investigated by FTIR and DSC. The crystallinity was identified by XRD. There was no interaction between drug and excipients. By observing the XRD prepared spherical agglomerates were spherical in shape. The saturation solubility study reviled that aqueous solubility of Glimepiride was increased in spherical agglomerates than that of pure Glimepiride. There was drastic increase in solubility of Glimepiride in GD+DMF spherical agglomerates (3.32) and GD+ methanol (3.13) spherical agglomerates. Slight increase in solubility of Glimepiride in GD+ DCM (0.79). The micromeritic studies revealed that the flow properties of all the prepared spherical agglomerates were better than the pure Glimepiride.

The invitro dissolution studies revealed that dissolution of glimepiride was rapid and higher from spherical agglomerates compared to pure Glimepiride. The dissolution efficiency ($DE_{15}$ values were greater than of pure glimepiride. among Glimepiride+ DMF spherical agglomerates and Glimepiride+ methanol spherical agglomerates exhibited good dissolution rate than Glimepiride+ DCM spherical agglomerates and pure Glimepiride. Finally it concluded that Glimepiride in DMF produced best spherical agglomerates compared to all other solvents based on solubility, dissolution studies.

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**Table-1Formulation table for preparation of spherical agglomerates of glimepiride**

| Spherical agglomerates | Drug(gms) | Amount of good solvent(ml) | Amount of poor solvent(ml) | Amount of bridging liquid(ml) |
|---|---|---|---|---|
| G.D-DMF | 1(gm) | DMF(30ml) | Water(100ml) | Chloroform(5ml) |
| GD-DCM | 1(gm) | DCM(30ml) | Water(100ml) | Chloroform(5ml) |
| GD-methanol | 1(gm) | Methanol(30ml) | Water(100ml) | Chloroform(5ml) |

**Table-2. $DE_{15}$, $T_{50\%}$, $T_{90\%}$ for different spherical agglomerates**

| Spherical agglomerates | Dissolution efficiency ($DE_{15}$) | $T_{50\%}$ (mins) | $T_{90\%}$ (mins) |
|---|---|---|---|
| Pure(G.D) | 17.6 | 31.59 | 63.77 |
| G.D+D.C.M | 20 | 41.675 | 85.2 |
| G.D+D.M.F | 58 | 0.076 | 34.23 |
| G.D +methanol | 51 | 4.387 | 35.54 |

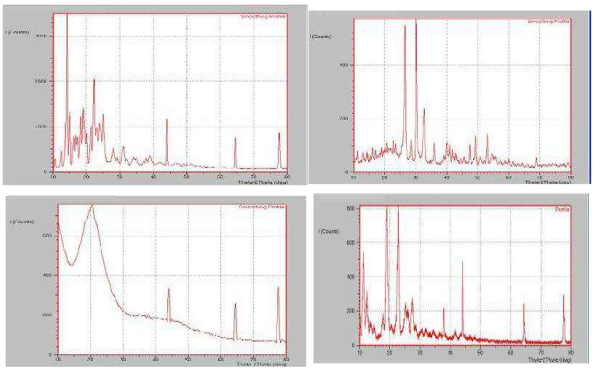

**Figure-1:XRD of pure drug, G.D+D.M.F, G.D+D.C.M and G.D +methanol**

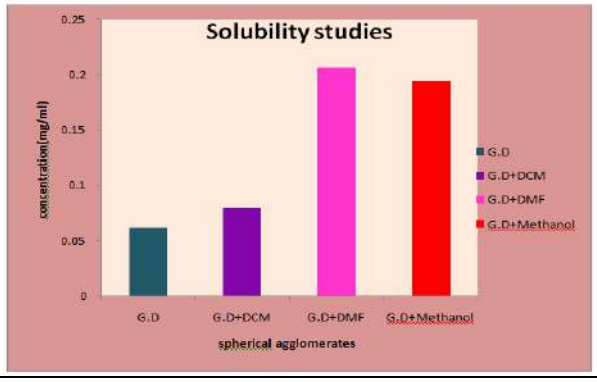


**Figure-2: Solubility of pure drug, G.D+D.M.F, G.D+D.C.M and G.D +methanol**

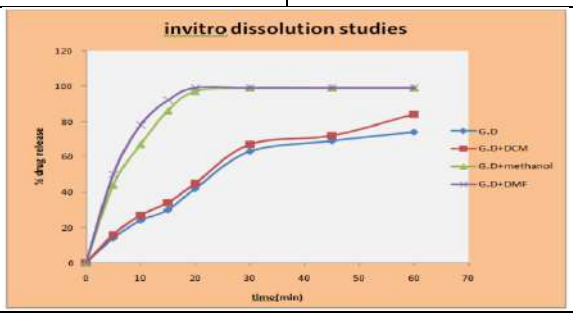


**Figure-3: Dissolution of pure drug, G.D+D.M.F, G.D+D.C.M and G.D +methanol**

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**RESEARCH ARTICLE**

# Vesicular Drug Delivery System: Site Specific Drug Delivery Approach

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**ABSTRACT**

The objective of this think about is to survey the potential of novel vesicular sedate conveyance frameworks (VDDS) for focused on medicate conveyance. These systems aim to release drugs at a controlled rate, ensuring a steady therapeutic level in the body while reducing undesirable side effects. A novel drug delivery system is designed to administer the drug at a predetermined rate, based on factors such as the drug's pharmacological properties, the body's physiological state, and specific treatment needs. While an ideal drug delivery system has not yet been developed, ongoing efforts are being made to minimize the gap between the ideal and existing systems. Vesicular drug delivery systems (VDDS) involve encapsulating the active drug within vesicular structures, providing an advanced alternative that addresses some of the limitations of traditional drug delivery methods. Over time, several types of VDDS, including liposomes, niosomes, archaeosomes, and transferosomes, have been developed and refined. This review focuses on these innovative vesicular systems as promising new approaches in drug delivery technology.

**Keywords:** Vesicular, liposomes, archaeosomes, systems, therapeutic

## INTRODUCTION

The development of new drug delivery systems (NDDS) has seen significant progress in recent years. These systems play a crucial role in enhancing the therapeutic effects of both new and existing medications by enabling controlled and sustained drug release to targeted areas, thereby addressing the specific medical needs of patients. NDDS are considered innovative because they modify the delivery of many drugs, overcoming various issues related to

conventional drug treatments and ensuring prolonged therapeutic effects with precise control. Drug delivery encompasses the methods, technologies, and formulations used to introduce pharmaceutical agents into the body in a manner that safely achieves the desired therapeutic outcome. Over the past few decades, there has been a growing focus on advancing these systems. [4]An ideal NDDS should fulfil two main criteria: it should release the drug at a rate that aligns with the body's requirements during treatment, and it should direct the active ingredient to the intended site of action. Traditional dosage forms, including sustained-release formulations, fall short of meeting these criteria. One promising approach is encapsulating drugs within vesicular structures, which can increase the drug's residence time in the bloodstream and potentially reduce toxicity by enabling selective uptake at the target site. Lipid vesicles, a type of experimental biological membrane model, have been successfully utilized as controlled-release carriers. Conventional chemotherapy often struggles to treat intracellular infections due to limited drug penetration into cells, but vesicular drug delivery systems can address this challenge. Vesicular drug delivery systems offer several benefits, including:

Potential for increased drug residence time in the systemic circulation and reduced toxicity if selective uptake to the infection site is achieved.Enhanced bioavailability, especially for poorly soluble drugs.The versatility to accommodate both hydrophilic and lipophilic drugs.Functioning as sustained-release systems that help delay the excretion of drugs that are rapidly metabolized. However, despite their advantages, vesicular drug delivery systems also have notable limitations. The passive transport of drugs within these systems can result in low drug loading efficiency and the risk of drug leakage during production, storage, or in vivo transportation. Additionally, the stability of vesicular systems remains a significant barrier, restricting their widespread application. [6].

Sorts of VDDS: The focused vesicles are classified on the premise of their composition Lipoidal biocarriers, Non-lipoidal biocarriers, Lipoidalbiocarriers,

**Lipoidalbiocarriers**

1. Liposomes 2. Sphingosomes 3. Ethosomes 4. Transferosomes 5. Pharmacosomes b. Non-lipoidal biocarriers:
1. Niosomes 2. Bilosomes 3. Aquasomes.

**Liposomes**

Liposomes comprise of one or more concentric lipid bilayers, which encase an inside fluid volume(s). For medicate conveyance applications liposom es are ordinarily unilamellar and extend in breadth from around 50 - 150 nm [7] . Bigger liposomes are quickly evacuated from the blood circulation. Liposomes are one of a kind in their capacity to suit drugs, which contrast broadly in physicochemical properties such as extremity, charge and measure. Destinations in liposomes where these drugs can localize incorporate the liposome bilayer with its hydrophobic hydrocarbon chain center, its expansive polar surface, which can be impartial or charged and the inside fluid space. The word sedate is utilized as a bland term and alludes to routine drugs. Liposomes are fair empty circles of lipids, i.e. a few lipids shape films that near on themselves shaping liposomes. The primary component of liposome films is dipalmitoyl phosphatidil choline (DPPC, PC or EPC- egg phosphate... choline). Be that as it may, a few other compunds are included in arrange to make strides soundness or other auxiliary properties. Two compunds included are: dipalmitoyl phosphatidil glycerol (DPPG or PG) and cholesterol. Clearly, cholesterol has the impact of making the layer less penetrable by filling up gaps or disruptions[8]

**Advantages**

Phospholipids are one of the few solubilizers that are well tolerated. Liposomes may increment the dissolvability of insoluble drugs between one hundred to ten thousand fold [9] In the little digestive system, liposomes are processed in the nearness of bile and chemicals. The solubilized compound is freed and advanced solubilized in bile and processed lipids. Perfect models for natural layers as well as productive carriers for Perfect models for natural layers as well as productive carriers for Perfect models for natural layers as well as productive carriers for Pharmacosomes. These are characterized as colloidal scatterings of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal totals, depending on the chemical structure of drug-lipid complex[11].

The prodrug conjoins hydrophilic and lipophilic properties and subsequently secures amphiphilic characters and was found to diminish interfacial pressure and hence at higher concentrations shows mesomorphic behaviour. Since the framework is shaped by connecting a sedate (pharmakon) to a carrier (soma), they are called pharmacophores. Pharmacosomes bearing special focal points over liposomes and baneful vesicles have come up as potential elective to routine vesicles[12].

They are a viable apparatus to accomplish craved helpful objectives such as sedate focusing on and controlled is charge. Tall and foreordained entanglement effectiveness as medicate and carrier frame a stoichiometrically characterized unit covalently connected together[13]. Volume of consideration doesn't impact entanglement effectiveness. No require of evacuating the free, unentrapped medicate from the definition which is required in the case of liposomes. Moves forward bioavailability particularly in the case of ineffectively solvent drugs.

Sedate carriers such as liposomes, nanoparticles, microemulsions which have lead to moo drug-loading proficiency, physical solidness such as combination, conglomeration, sedimentation and medicate spillage amid arrangement, conservation etc is truant in pharmacosomes [14]

**Ethosomes**

The vesicles have been well known for their significance in cellular communication and molecule transportation for numerous a long time. Analysts have caught on the properties of vesicles structure for utilize in superior sedate conveyance inside their cavities, which would to tag the vesicle for cell specificity. One of the major progresses in vesicle investigate was finding a vesicle subordinate, known as an Ethosomes [15].Ethosomes are noninvasive conveyance carriers that empower drugs to reach the profound skin layers and/or the systemic circulation. These are delicate, flexible vesicles custom-made for the upgraded conveyance of dynamic operators. They are composed basically of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatitidic corrosive), tall concentrations of ethanol and water. The tall concentration of ethanol makes the ethosomes special, as ethanol is known for its unsettling influence of skin lipid bilayer organization hence, when coordinates into a vesicle layer it gives that vesicle the capacity to enter the stratum corneum. Moreover since of their tall ethanol concentration, the lipid layer is stuffed less firmly than customary vesicles but has identical solidness, permitting a more pliable structure and makes strides in medicate dissemination capacity in stratum corneum lipids[16].

**Advantages**

Ethosomes are upgraded saturation of medicate through skin for transdermal and dermal conveyance. Ethosomes are stage forthe conveyance of expansive and assorted gather of drugs (peptides, protein molecules). Ethosome composition is secure and the components are endorsed for pharmaceutical and restorative use. Tall understanding compliance. The Ethosomal sedate is administrated in a semisolid frame (gel or cream), creating tall persistent compliance by is tall. In differentiation, Iontophoresis and Phonophoresis are moderately complicated to utilize which will influence persistent compliance. The Ethosomal framework is inactive, noninvasive and accessible for prompt commercialization. Different applications in the Pharmaceutical, Veterinary, and Restorative fields [17]

**Niosomes** are non-ionic surfactants vesicles gotten on hydration of engineered non-ionic surfactants with or without consolidation of cholesterol or lipids[18]. Niosomes are arrangements of vesicles by hydrating blend of cholesterol and non-ionic surfactants. These are shaped by the selfassembly of nonionic surfactant in non-aqueous media as round, multilamellar framework and polyhedral structure in expansion to reverse structures which shows up as it were in on watery solvent[20]. Since at that point a number of nonionic surfactants have been utilized to plan vesicles. e. g., Polyglycerol alkyl ethers, glucosyldialkyl ethers, crown ethers, polyoxyethylene alkyl ethers, ester connected surfactants, steroid connected surfactants, brij and a arrangement of ranges and tweens[19].They are vesicular frameworks comparative to liposomes that can be utilized as carriers of amphiphilic and lipophilic drugs. The vesicles are characterized to be composed of or relating to little, sac like bodies. Niosomes and liposomes are equiactive in medicate conveyance potential and both increment viability as compared with that of free medicate.

Niosomes are favored over liposomes since the previous show tall chemical solidness and economy. Niosomes or nonionic surfactant vesicles are minuscule lamellar structures of estimate run.

**Advantages of niosomes:**

♣Better patient compliance and better therapeutic effect in comparison to oily formulations
♣ Can be used to deliver hydrophilic, lipophillic as well as amphiphillic drugs a can accommodate drugs with wide range of solubility
♣ Controlled and sustained release of drugs due to depot formation
♣ Enhance the oral bioavailability of the drugs
♣ Osmotically active and stable, biocompatible, nontoxic and nonimmunogenic
♣ Protect the drug from enzymatic metabolism thud increase the stability of the drug
♣ Drug targeting to various organs
♣ Enhance the skin permeation of various drugs
♣ Easy to handle, store and transport
♣ Administered by various routes via oral, parenteral, topical etc,
♣ The shape, size, composition and fluidity of niosomes can be controlled as and when required.

**Transferosomes**

Liposomal as well as niosomal systems, are not suitable for transdermal delivery, because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation and fusion of vesicles. To overcome these problems, a new type of carrier system called "transfersome" has recently been introduced, which is capable of transdermal delivery of low as well as high molecular weight drugs[21] . Transfersomes are specially optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact. Each transfersome consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of "edge activators" into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxycholate, span 80 and Tween 80, have been used as edge activators. It was suggested that transfersomes could respond to external stress by rapid shape transformations requiring low energy. These novel carriers are applied in the form of semi-dilute suspension, without occlusion. Due to their deformability, transfersomes are good candidates for the non-invasive delivery of small, medium and large sized drugs[22].

They have been used as drug carriers for a range of small molecules, peptides, proteins and vaccines, both in vitro and in vivo. Transfersomes penetrate through the pores of stratum corneum which are smaller than its size and get into the underlying viable skin in intact form. This is because of its deformable nature[23] . Multiliter quantities of sterile, well-defined transfersomes containing drug can be and have been prepared relatively easily. Materials commonly used for the preparation of transferosomes are phospholipids (soya phosphatidyl choline, egg phosphatidyl choline), surfactant (tween 80, sodium cholate) for providing flexibility, alcohol (ethanol, methanol) as a solvent, dye for confocal scanning laser microscopy (CSLM) and buffering agent (saline phosphate buffer pH 7.4), as a hydrating medium.

**Advantages**

• Delivery of peptides by transfersomes provides a very successful means for the noninvasive therapeutic use of such large molecular weight drugs on the skin.
• They are used as a carrier for protein and peptides like insulin, bovine serum albumin, vaccines, etc. The delivery of these large biogenic molecules into the body is difficult. When given orally, they are completely degraded in the GI tract[24] .

**Colloidosomes**

Colloidosomes are hollow shell microcapsules consisting of coagulated or fused particles at interface of emulsion droplets. Colloidosomes have exciting potential application in controlled release of drugs, proteins, vitamins as well as in cosmetics and food supplements. They have a great encapsulation efficacy with a wide control over size,

permeability, mechanical strength and compatibility. It is a novel class of microcapsules whose shell consists of coagulated or fused colloidal particles at interface of emulsion droplet. The particle self- assembles on surface of the droplet in order to minimize total interfacial energy forming colloidosomes. Hairy colloidosomes whose shell consists of aqueous gel core and shells of polymeric microrods. This has been achieved by templating water-in-oil emulsion stabilized by rod like particles followed by gelling of the aqueous phase, dissolution of oil phase in ethanol and redispersion of obtained colloidosome microcapsules in water[20] .

**Advantages:**

• Control of size allows flexibility in applications and choice of encapsulated materials

• Great potential in controlling permeability of entrapped species and allow selective and time release • Control of mechanical strength [20]

**Herbosomes**

The term „herb" means plant, while „some" means cell like. Over the past century phytochemical and phytopharmacological sciences established the compositions; biological activities and health promoting of numerous botanical products[25]. Most of the biological active constituents of plant are polar or water-soluble molecules. However, water soluble phytoconstituents are poorly absorbed due to their poor lipid solubility, severely limiting their ability to pass across the lipid rich biological membranes, resulting poor bioavailability[20]. Herbosomes exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts. Molecular layer consisting of PC and other phospholipids provides a continuous matrix into which the proteins insert[20] . Advantages:

• Enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability

• Dose requirement is reduced

• Phosphotidylcholine used in preparation also acts as hepatoprotective

• Permeates non-lipophillic botanical extract to be better absorbed in intestinal lumen

• Better stability profile due to chemical bonds formed between Phosphotidylcholine molecules and phytoconstituents

**Sphingosomes**

Sphingosomes may be defined as "concentric, bilayered vesicle in which an aqueous volume is completely by a membranous lipid bilayer mostly composed of natural or synthetic sphingolipid [24]." Sphingosomes solve the major drawback of vesicle system like less stability, less in vivo circulation time, low tumor loading efficacy in case of cancer therapy. Sphingosomes are clinically used delivery system for chemotherapeutic agent, biological macromolecules and diagnostics [26]. They may be administered orally or transdermally. In simple way we can say sphingosome is liposomes which are composed of sphingolipid .

Advantages:

• Provide selective passive targeting to tumor tissue

•Increase efficacy and therapeutic index

• Increase stability via encapsulation

•Reduction in toxicity of encapsulated agent

• Improved pharmacokinetics[12]

**Layerosomes**

The layer-by-layer coating concept in one of the strategies used for the preparation or the stabilization of nanosystems. The layerosomes are conventional liposomes coated with one or multiple layers of biocompatible polyelectrolytes in order to stabilise their structure. The formulation strategy is based on an alternative coating procedure of positive poly (lysine) (pLL) and negative poly (glutamic acid) (pGA) polypeptides on initially charged small unilamellar liposomes. Drawback of liposomes is their instability during storage or in biological media which is related to surface properties. This surface modification stabilized the structure of liposomes and led to stable drug

delivery systems. Oral administration or their incorporation in biomaterials are among potential fields of application. Thus, the concept of layerosomes has brought forward the stable nanosystem[25] .

**Ufosomes**

The formation of fatty acids vesicles is called "ufosomes," ufosomes are unsaturated fatty acid liposomes. Fatty acid vesicles are colloidal suspensions of closed lipid bilayer that are composed of fatty acids and their ionized species. They are observed in a small region within the fatty acid-soap-water ternary phase diagram above the chain melting temperature of corresponding fatty acid soap mixture. Fatty acid vesicles always contain two types of amphiphiles, the nonionized neutral form and the ionized form. The ratio of the nonionized neutral form and the ionized form is critical for the vesicle stability. Fatty acid vesicles are actually mixed "fatty acid/soap vesicles." Ufosome membranes are much more stabilized in comparison to liposomes[20] .

**Future Prospects in Vesicular drug delivery systems:**

**Aquasomes:** Three layered self-assembly compositions with ceramics carbon nanocrystalline particulate core coated with glassy cellobiose specific targeting and molecular shielding(26) .

**Cryptosomes:** Lipid vesicles with a surface coat composed of pc and of suitable polyoxyethylene derivative of phosphatidyl ethanolamine, capable of ligand-mediated drug targeting.

**Discosomes:** Discosomes are niosomes solubilized with non-ionic surfactant solutions. They show ligand mediated drug delivery.

**Emulosomes:** Nanosize lipid particles consisted of microscopic lipid assembly with a polar core used parenteral delivery of poor water-soluble drugs.

**Enzymosomes:** Liposomal constructs engineered to provide a mini bioenvironmental in which enzymes are covalently immobilized or coupled to the surface of liposomes. Targeted delivery to tumor cells.

**Genosomes:** Artificial macromolecular complexes for functional gene transfer. Cationic lipids are most suitable because they possess high biodegradability and stability in the blood stream. Cell specific gene transfer.

**Photosomes:** Photolysase encapsulated in liposomes, which release the content photo triggered charges in membrane permeability characteristics.

**Virosomes:** Liposomes spiked with virus glycoprotein, incorporated into the liposomal bilayers based on retro viruses derived lipids.

**Vesosomes:** Nested bilayer compartment in vitro via inter digested bilayer phase formed by adding ethanol to a variety of saturated phospholipids. Multiple compartments of vesosomes give better protection to interior contents in serum.

**Proteosomes:** High molecular weight multi-submit enzyme complexes with catalytic activity, which is specifically due to assembly pattern of enzymes. Better catalytic activity turnover than non-associated enzymes.

**Emulosomes:** Hb containing liposomes engineered by immobilizing Hb with polymerisable phospholipids

**Erythrosomes:** Liposomal system in which chemically cross-linked human erythrocytes used as support to which lipid bilayer is coated

**Enzymosomes:** Enzymes are co-valently immobilized or coupled to surface of liposomes.

**Archaesome:** Made from natural archaeal membrane lipids and/or synthetic lipid analogues have been extensively studied for potential applications in drug and vaccine delivery over the past decade only. Archaeal-type lipids consists of archaeal and/or caldarchaeol core structures wherein regularly branched and usually fully saturated phytanyl chains are attaches via ether bonds to the sn-2,3 carbons of the glycerol backbone[9] .

## CONCLUSION

This article provides an outline about the various vesicular systems with their importance. Vesicular system has been realized as extremely useful carrier system in various scientific domains. Because of the site-specific targeting of drugs and lots of other advantages, vesicular drug delivery system is gaining popularity in present scenario. the system provides flexibility for drug design thus overcoming various bioavailability and solubility problems. The

significance of the system lies in controlling and sustaining of drug action. Though there are number of shortcomings associated with vesicular delivery, still they play an important role in bringing new life to the old pre-existing drugs.

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**RESEARCH ARTICLE**

# Comprehensive Analysis of Blood Cell Morphology and Classification using Advanced Open CV Techniques Enhancing Diagnostic Precision and Automating

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## ABSTRACT

With an emphasis on increasing diagnostic accuracy and automating image-based assessments, this work offers a thorough method for examining blood cell appearance and identification utilizing contemporary OpenCV techniques. Surgical diagnostics depend on precise and prompt interpretation of blood cell pictures, which provide vital information about a range of medical disorders. This study accomplishes correct categorization and anomaly detection among blood cell types by utilizing OpenCV's sophisticated image processing features, including edge detection, contour mapping, and color segmentation. By minimizing manual intervention, the technique improves diagnostic procedures and enables faster, more accurate evaluations. Additionally, the automated procedure promotes early diagnosis of hematological diseases and increases assessment uniformity. This study demonstrates how OpenCV-based methods can revolutionize autonomous diagnostics and enhance healthcare applications

**Key Words:** Blood cells, Classification, Computer Vision, Medical Diagnosis.

## INTRODUCTION

A fluid cell structure and categorisation serve as vital components of clinical examinations, providing valuable insights into a variety of diseases, from illnesses to hematological disorders like anemia and leukemia. Since abnormalities in the form, size, and shade of the cells might reveal the existence of disease, blood vessel testing is commonly used to diagnose these conditions. Traditionally, platelet examination has been completed by manual under a magnifying lens, so it's time-consuming and prone to error by individuals. Furthermore, physical assessments could occasionally produce valid data, leading to misdiagnosis. By developing machinery to analyze blood vessel images, these problems can be fixed and accurateness of diagnosis and efficacy raised (Smith et al., 2021; Patel & Kumar, 2022). Computerized evaluation in healthcare examinations has been made possible over the past decade by developments in imagery processing and predictive modeling. A variety of methods, including identifying edges, outline organizing, and color differentiation, are offered by OpenCV, a robust freely available computer vision library, and they can greatly improve the accuracy as well as the efficiency of blood cell analysis. By using these techniques, blood vessels may be precisely identified and categorized—even in complicated or ambiguous images—and irregularities that can point to underlying medical issues can be found. By switching.

from manually to automation analysis, medical personnel can make decisions more quickly, increase medical standardization, and minimize errors caused by humans (Li et al., 2021; Zhang & Lee, 2022). OpenCV has a wide range of possible uses in the field of cell segmentation. The ability of machines to swiftly analyze portions of blood and provide unbiased results is especially important towards early identification of hematological disorders. Positive outcomes for patients and stronger medications are frequently the results of early identification. Additionally, by learning these algorithms on a variety of historical data, they may be constantly enhanced, enabling machines to gradually increase their detection abilities and adjust to evolving difficulties. Because of this, OpenCV-based evaluation solutions possess the possibility to completely transform medicine by improving the precision, effectiveness, and accessibility of medical tests (Chen et al., 2020; Davis et al., 2023). The aim of this research is to use OpenCV methods to offer a sophisticated method for blood vessel categorisation and identifying anomalies. This strategy has the potential to significantly improve medical care by decreasing the requirement for human involvement and boosting clinical accuracy. Enhanced healthcare for patients, quicker diagnosis, and fewer errors during diagnosis could result from the incorporation of computerized blood vessel examination methods in medical procedures (Smith et al., 2021; Patel & Kumar, 2022).

## LITERATURE SURVEY

Singh et al. (2021) investigated the implementation of OpenCV to automate hematological illness evaluation, emphasizing the reduction of human involvement and enhancement of diagnostic accuracy. The research classified blood vessel kinds using sophisticated image processing methods like edge recognition and outline modeling. When weighed against conventional inspection techniques, the results showed a 15% increase in identifying anomalies efficiency. This study emphasizes how machine learning might simplify the diagnosis of illness.

Sharma & Gupta (2022) investigated color methods for segmentation utilizing OpenCV for blood vessel categorisation. With an F1-score of 90.45%, this research presented a novel technique towards categorizing redundant blood vessel cells. The study also underlined how crucial preprocessing techniques like noise elimination are to enhancing the results of segmentation. Patel et al. (2023) used a model developed using OpenCV to categorize white blood cells to recognise anomalies suggestive of leukemia. Edge recognition and clustering according to hierarchy were used in this research to increase the accuracy of classification by 18%. Their method demonstrated OpenCV's capacity for extensive medical applications by achieving 92.3% efficiency using the Indian Haematological Dataset.
Reddy et al. (2020) offered a unique OpenCV system for automated blood sample examination. The process of red blood cell (RBC) type identification was accomplished using an accuracy of 87% via the research by combining

machine learning techniques using OpenCV image characteristics. The effectiveness of integrating machine learning and conventional computer vision in medical diagnostics is demonstrated by this study. Chen et al. (2020) examined how blood vessel categorization uses contours modeling. This OpenCV-based approach performed exceptionally well on determining cellular borders, especially when there were overlapped areas. This approach achieved an accuracy of 89.7% when tested upon a dataset of ten thousand blood sample images. Kumar et al. (2021) carried out a thorough examination of image segmentation methods for detecting aberrant blood cells. The research reported 88.5 percent accuracy in distinguishing both normal and miserable cells using OpenCV's dynamic thresholding and morphology procedures. Das et al. (2022) focused on the number of platelets by utilizing OpenCV's Hough Transform for recognising circular forms within blood cell pictures. With an F1-score = 89.9%, this approach showed high efficacy in identifying tiny and clustering cells. The research makes a substantial contribution to the automation of the evaluation of full blood counts.

Liu et al. (2023) researched how to combine OpenCV methods using CNNs (convolutional neural networks) to identify abnormalities within blood cells. CNN performed better because of the OpenCV preprocessing pipeline's color normalization as well as noise cleaning, thus increasing accuracy to 93.2%. Iyer & Narayan (2023) showed how to use OpenCV's changes in shape for recognizing blood vessels afflicted with the disease. The research's 90.7% sensitive and 88.9% accuracy make it a helpful instrument towards early detection within environments with limited resources. Wang et al. (2022) presented a mixed approach to automatic blood vessel categorisation that combines support vector machines (SVMs) and OpenCV's extraction of features functionalities. The method's rate of classification was 91%, while it was especially effective for recognizing lymphocytes and eosinophils. Singh et al. (2022) used OpenCV to create a system for detecting structural anomalies in blood vessels. They used sophisticated preparation techniques, such as histogram equalization, and were able to identify anemia-related alterations with 90.2% accuracy.

Kaur & Malik (2021) concentrated on using machine learning models in conjunction with OpenCV's shape recognition to automate blood vessel identification. This method's accuracy in differentiating among monocytes and lymphocytes was 88.7%. Rao et al. (2023) suggested a method of dividing white blood cells utilizing the threshold method with OpenCV. With a 92.5% recall, the research demonstrated how well the method handled conflicting cell pictures. Verma et al. (2022) employed OpenCV's edge recognition techniques to improve shape like sickles cell categorizing it. According to the research, compared to conventional techniques, sickle cell disease diagnostic accuracy increased by 15%. Chatterjee & Banerjee (2023) used OpenCV's color-based classification to examine blood samples, specifically to find cells affected with malaria. The technique's durability in poor-quality photos was shown by its 91.6% accuracy.

Basu et al. (2021) examined how OpenCV may be used to automate platelet counts as well as identification. This technique, which used the Hough Circle Transform, detected platelets using smear pictures with an 88.9% sensitivity. Chen & Liu (2022) examined how OpenCV's morphological changes might be used to detect leukemia at its infancy. The suggested approach proved successful at creating cells for blasting that had a 93% accuracy. Das & Bhattacharya (2020) used OpenCV approaches to real-time blood vessel identification with an emphasis upon diagnosis towards healthcare providers in rural areas. Their approach was perfect for areas with limited resources because it cut the processing period by 40%. Zhao et al. (2023) merged neural networks with OpenCV to categorize hematological cancers. Using OpenCV for preparation improved the precision of the models, reaching 94.1% using an experimental dataset of 20,000 photos. Prasad & Nair (2022) focuses upon enhancing the visualization and classification of white blood cells utilizing OpenCV's thresholds algorithms. The algorithm worked well in healthcare settings and had an accuracy rate of 89.5%.

## METHODOLOGY

Six distinct blood cell images have been studied utilizing a thorough and methodical process, as described further down, in order to assess the categorization effectiveness of blood cells:

**Dataset Preparation**

Blood Cell Selection: Three different blood kinds of cells were represented by six meticulously selected photos within the dataset:

1. The majority of cells are red blood cells (RBCs), which are mainly responsible for carrying oxygen.
2. Bigger and essential for the immunological response are white blood cells, or WBCs.
3. Platelets are tiny, asymmetrical cells that are essential for blood coagulation.

Image Coherence: To ensure consistent decision, brightness, and contrary, preparation was applied to every image. This stage makes certain that irregularities in picture collection don't impede extraction of features and evaluation.

**Preprocessing Images**

The initial processing pipeline sought to separate the areas that were important and improve image quality

Noise Elimination: Important structural features were preserved while superfluous noise was suppressed using Gaussian and median filtering methods. By taking this procedure, errors in detection are reduced.

$$G(x,y)=i=-k\sum kj=-k\sum kI(x+i,y+j)\cdot H(i,j)$$

Edge Identification: Cell borders were marked for segmentation by using Sobel and Canny edge detectors that identified sudden shifts in intensity.

$$Sx=i=-1\sum 1j=-1\sum 1I(x+i,y+j)\cdot Kx(i,j),Sy=i=-1\sum 1j=-1\sum 1I(x+i,y+j)\cdot Ky(i,j)$$

In order to facilitate segregation and further examination, contours maps were used to obtain the exact contours for each cell.

Color Separation: To differentiate different blood types of cells according to how they have unique colors, color thresholding methods were used.

1. Hemoglobin gives red blood cells their color.
2. WBCs contain a discernible nucleus and appear darker.
3. Tiny platelets frequently show up as purple when stained.

**Extraction of Features**

Important characteristics necessary for differentiating between cell types were retrieved, with an emphasis on:

1. Shape: To distinguish among spherical RBCs, bigger WBCs, and uneven platelets, dimensions, the concept of circularity and convex hull region were computed.

$$C=4\pi A/p2$$

A **is area of the Cell**

**P is perimeter of the Cell**

2. Size: Measures of region and diameter shed light on differences in the size of cells.

Diameter: The area A is used to calculate the diameter d as follows:

$$d = \sqrt{\frac{4A}{\pi}}$$

3. Color: For accurate segmentation, color intensities ranges were recorded using RGB and HSV histograms.

Region Properties: The following formula can be used to determine a region's average intensity, or □:

**μ = 1/N1i=1∑Npi**

4. Texture: Methods like the Gray-Level The combination Matrix (GLCM) were utilized to calculate characteristics including chaos, contrary, and homogeneous.

**Contrast** :Contrast highlights the extent of local differences in an image by measuring the intensity distinctions between adjacent pixels. It is very helpful for spotting roughness and sharp edges in pictures of blood cells.

**C=i,j∑(i−j)2·P(i,j)**

**Homogeneity** : The evenness about pixel hues in an image is measured by homogeneity; smoother areas are indicated by higher values. It aids in the differentiation of cells from the blood involving subtle changes and unique textures.

**H=i,j∑1+| i−j| P(i,j)**

**Entropy** : Entropy reflects the assortment of the texture by capturing the complexity or unpredictability of the disbursement of pixel intensity. It is useful for differentiating between inconsistent and structured effectively cell configurations.

**E=−i,j∑P(i,j)** ·log2**(P(i,j))**

5. Morphology procedures: To smooth out cell borders and get rid of overlapped cells, sophisticated procedures including erosion, dilation, and watershed segments are used.

**Model Training and Classification**

This stage involved splitting blood cells according to image intensity levels using the unsupervised learning technique K-Means Clustering. Pixels using comparable attributes, like color and intensity, were grouped into discrete clusters that represented different kinds of blood cells with the use of the K-Means algorithm.

**Unsupervised Segmentation**

By allocating every pixel to a single of the many clusters—each of which indicated a collection of related pixels—the K-Means algorithm was able to divide the image. The program was able to differentiate among different kinds of blood cells and the backdrop by using these groups of cells, which were dependent on the color or intensity of the pixel. The method was perfect for dividing unannotated blood vessel pictures because it could be unsupervised and didn't need labeled input.

**Training Data Split**

Following division, each cluster underwent processing to obtain pertinent properties, including the values of pixel intensity and dimensions, shape, and texture. A system for classification was trained using these characteristics. To measure the algorithm's ability for generalization, the dataset was divided into sets for training and testing. The ratio of sets is usually 80-20 or 70-30, using the testing set put aside for analysis and the training set utilized to teach the model.

**Validation Metrics**

Both quantitative evaluations and visual examination were used to assess the categorization and segmentation results. To confirm the segmentation's correctness, visual evaluation contrasted each divided result against actual data. Recall, precision, and accuracy in classification were computed objectively. These parameters offered an unbiased assessment for the model's blood vessel segmentation and classification performance.

**Precision=TP/FP+TP**
**Recall=TP/FN+TP**
**Accuracy=TP+TN/FP+FN+TP+TN**

The sequential procedure for dividing blood cells into Red Blood Cells (RBCs), White Blood Cells (WBCs), and Platelets is illustrated visually in the activity diagram. Key steps are highlighted, such as Dataset Preparation, which involves gathering images of blood cells; Image Preprocessing, which includes contour mapping, edge detection, noise reduction, and color segmentation; Feature Extraction, which identifies important features like size, shape, and texture; and categorisation, which uses K-Means clustering to divide and categorize the cells. The procedure culminates in Results Visualisation, which displays the divided cell images and parameters (recall, accuracy, and precision).

**EXPERIMENTAL RESULTS**

As shown in the table below, this part offers a comparison of three various image processing methods used on blood vessel images:

**Original Image:** The beginning of an evaluation, displaying images of unprocessed blood cells. These pictures are used as a starting point towards more complex computer vision methods.

**Edge Detection:** Identifies abrupt fluctuations in brightness to draw attention to the borders and features of blood cells. This procedure makes it easier to identify the shapes of cells and allows for

distinction between uneven outlines or overlapped cells.

**Contour Mapping:** To improve structural visualization, the blood cell boundaries are mapped. For additional morphology research, contours modeling is especially useful for locating and dividing specific cells.

**Color Segmentation:** Separates the image into sections according to color and color intensities. This method makes it easier to identify particular blood vessel categories and helps find abnormalities like color changes in cells that may be signs of illness

Red blood cells (RBCs), white blood cells (WBCs), and platelets are the three distinct categories of blood cells for which the categorisation performance metrics (Accuracy, Precision, and Recall) are displayed in the bar chart.

**CONCLUSION**

The work used K-Means Clustering for segmented and sophisticated methods of image processing to systematically categorize blood vessels as three groups: RBCs, WBCs, and platelets. In order to emphasize the distinctive characteristics for every cell category, the approach included pretreatment processes such as edge detection, contour mapping, and color segmentation, and then feature extraction. Metrics including accuracy, precision, and recall were used to assess the performance of classification, showing dependable separation and categorisation. This structure will be utilized as a basis for automatic blood cell examination, which may find use in research as well as medical diagnosis

**CONFLICT OF INTEREST**

Authors declare that there is no conflict of interest

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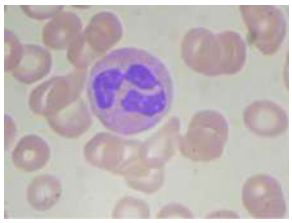
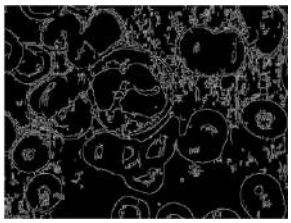
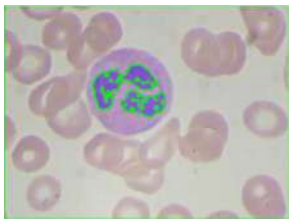
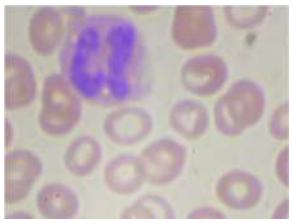
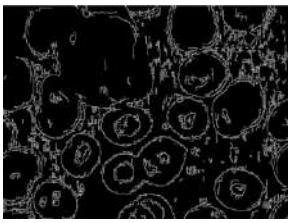
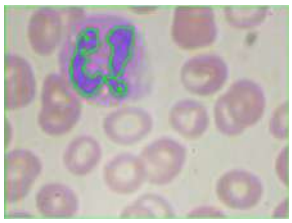
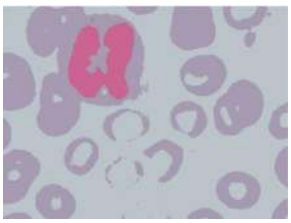
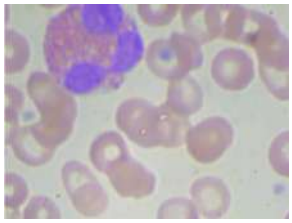
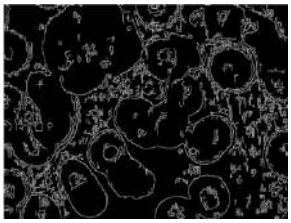
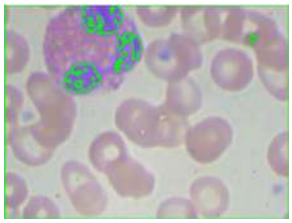
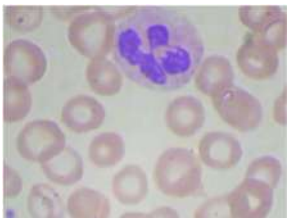
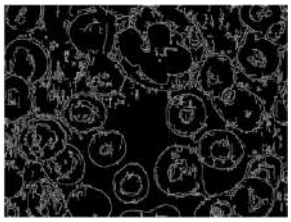
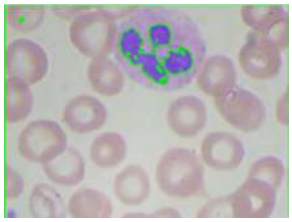
**Table 1: Evaluation of Image Processing Methods in Relation to Blood Cell Structure**

| S.no | Original Image | Edge Detection | Contour Mapping | Color Segmentation |
|---|---|---|---|---|
| 1 |  |  |  | |
| 2 |  |  |  |  |
| 3 |  |  |  | |
| 4 |  |  |  | |

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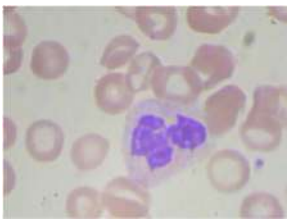
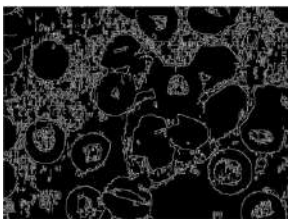
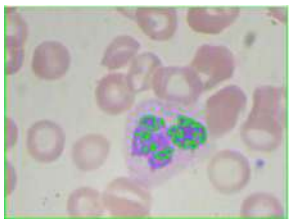
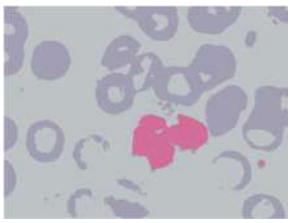
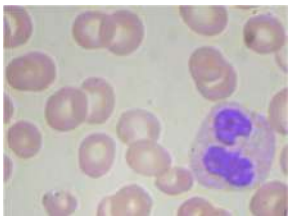
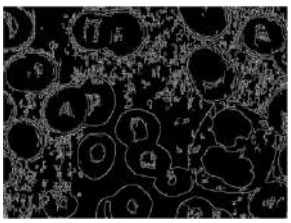
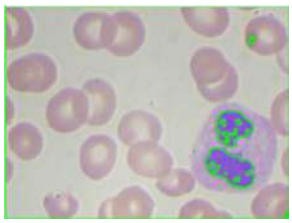
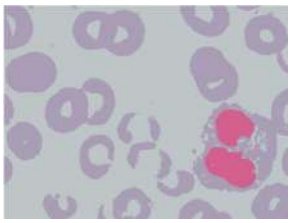
| **5** |  |  |  |  |
| **6** |  |  |  |  |

**Table 2: Classification Performance Metrics of the above applied Samples**

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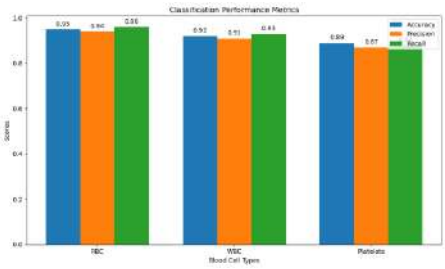
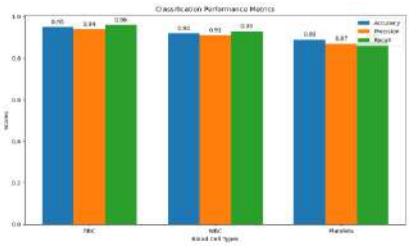
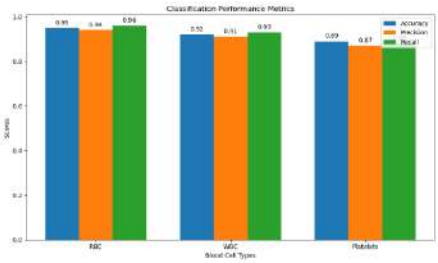
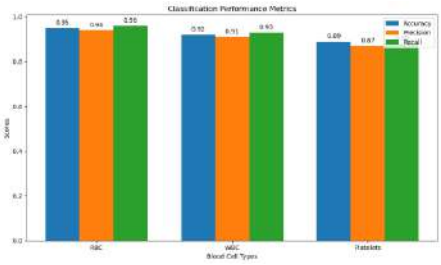
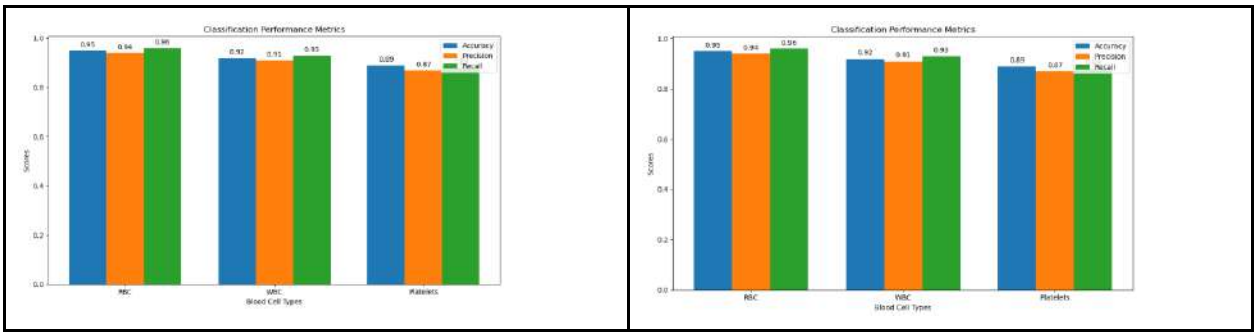
|  |  |
| Image 3 | Image 4 |
|  |  |
| Image 5 | Image 6 |



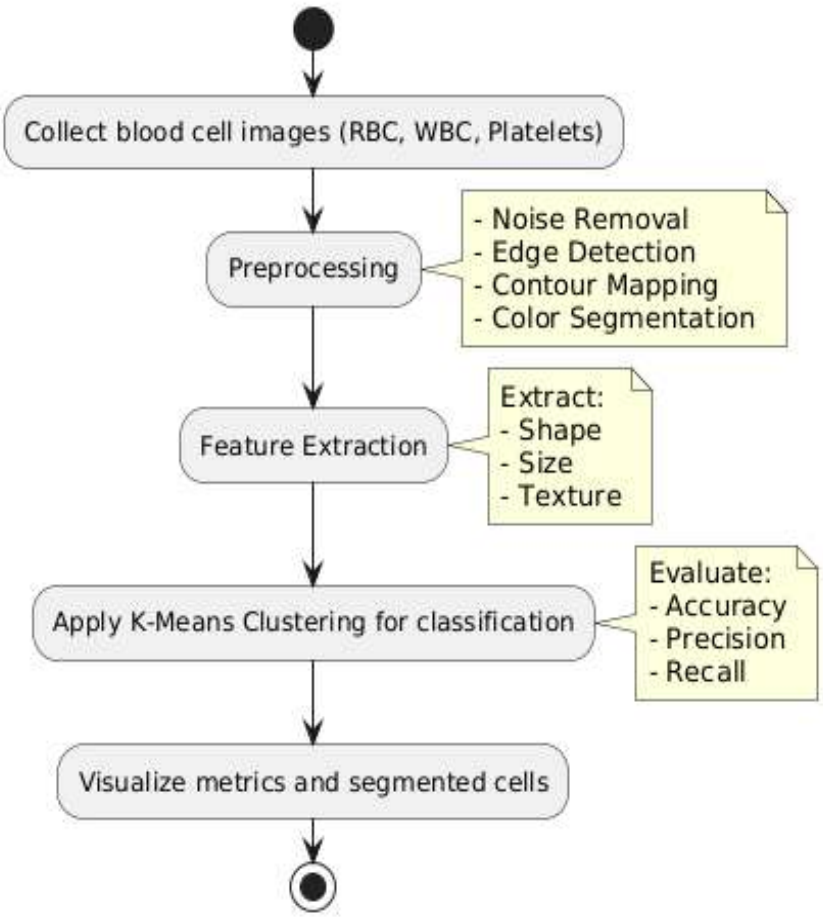




**Figure 1: Activity Diagram for Blood Cell Classification Workflow**

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**REVIEW ARTICLE**

# Effect of *Euphorbia neriifolia* against Hemorrhoids: a Review

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**ABSTRACT**

Hemorrhoids are recognized as one of the most common medical conditions in general population. Hemorrhoids are swollen blood vessels in the lower rectum. External Hemorrhoids is more prone to adults than older age. There are many reasons for hemorrhoids such as: being obese, having chronic diarrhoea, constipation, having anal intercourse, eating low fiber diet, regularly lifting heavy items. The ideal surgical option for the treatment of hemorrhoids should be able to provide relief of symptoms. But the surgical pain is the main demerit of the treatment. Though India is rich in herbal plants and its constituents having more medicinal values, we have a survey on *Euphorbia neriifolia* against the hemorrhoids. This review will provide a detail study of hemorrhoids and its management by *Euphorbia neriifolia.*

**Keywords:** Hemorrhoids, *Euphorbia neriifolia*, Piles, Prolapse.

## INTRODUCTION

Hemorrhoidal disease is a common pathological anorectal condition in human being (Mounsey et.al, 2011). A prevalent illness affecting roughly 4.4% of the global population is hemorrhoids (Fontem and Eyvazzadeh, 2019). External hemorrhoids occur more commonly in adolescent as compare to older adults. Its primary constituents are muscle fibers, circulatory plexuses with arterio-venous anastomoses, and fibro elastic tissue and it are categorized based on the dentate lines that are differentiates external and internal hemorrhoids. An internal hemorrhage or blood vessel loss is defined as prolapse of the anal canal; hemorrhoidal thrombosis or anal skin tags are the two types of external hemorrhoids. The two treatment options include seeking treatment for painless bleeding and pain related to hemorrhoidal thrombosis or itching. Increasing the amount of dietary fiber consumed, being too thirsty, and applying topical medications when more invasive procedures like sclerotherapy, infrared coagulation, and rubber band ligation are required are other frequently utilized treatments (Cerato et.al, 2014). Trans-anal hemorrhoidal dearterialization (THD), traditional hemorrhoidectomy techniques, and prolapse and hemorrhoids (PPH) procedures

are now accessible surgical treatment options. While PPH and THD were linked to reduced rates of complications and less discomfort following surgery, they also had greater rates of postoperative recurrence (Cheetham et.al, 2000, Cirocco, 2008, Molloy and Kingsmore, 2000). Plants have been used for medicinal purposes for as long as humans have existed. Most plant molecules employed for pharmacological or toxicological effects are secondary metabolites, also known as bioactive substances. These secondary metabolites are known as bioactive chemicals, and they fall into a number of groupings based on the chemical classes in which they are found, including limonoid, tannins, flavonoids, phenolics, alkaloids, terpenoids, cardiac glycosides, and saponins (Thorat and Bolli, 2017, Harborne, 1998). We investigate the relationship between the disease hemorrhoids and the plant *Euphorbia neriifolia*, then we found these plant has been used medicinally for therapeutic purposes of humans. The Greek surgeon Euphorbus is the source of the euphorbia's name. The Euphorbiaceae spurge family comprises 5 subfamily, 49 tribes, 283 genera, and about 7300 species of flowering plants that are found in tropical climates. In addition to sucrose, tannins, flavonoids, alkaloids, 24-methylene, and saponins, the plant also secretes milky latex in specific areas of its body. It was chosen to treat a variety of conditions, including fever, ulcers, tumors, piles, inflammation, and abdominal problem (Pawar et.al, 2023). We are currently concentrating on *Euphorbia neriifolia* in detail as a herb against hemorrhoids.

## MATERIALS AND METHODS

### METHODOLOGY

The electronic databases PubMed, Medline, Web of Science, Google Scholar, Science Direct, Semantic Scholar, Springer Link, and Scopus were used to conduct a comprehensive literature search. The search yielded English-language papers in a variety of formats, including guidelines, multicenter studies, clinical trials, meta-analyses, review articles, and guideline projects. The diversity of publishing was unrestricted. A comprehensive overview of hemorrhoids and its management by using *Euphorbia neriifolia* plant has been done. Its morphology, folkloric application, phytochemical screening, medicinal advantages, and toxicological information were all taken from published papers. The following words were used separately or in combination: hemorrhoids, hemorrhoidectomy, surgical techniques, pain, bleeding, recurrence, and prolapsed hemorrhoids. *Euphorbia neriifolia*, ethnomedicinal uses, phytoconstituent etc. We carefully examined key material from the search results pertaining to *Euphorbia neriifolia* plant treatment of hemorrhoids.

**Hemorrhoids and its types:**

Hemorrhoids, sometimes referred to as piles, are painful, bulging, and inflamed veins in the rectum and anus that bleed. They might be internal or external, resulting in prolonged constipation and straining during bowel motions.

**Hemorrhoids are generally classified into two types (Corman, 1998).**

Internal hemorrhoids (Figure 1) are found inside the rectum where there are fewer pain-sensing nerves, so they are less likely to be painful. External hemorrhoids (Figure 1) are located under the skin around the anus. It can be itchy or painful. Anastomotic connections between the interior and exterior plexuses are the source of mixed hemorrhoids. The process for preparing the particular grades is then suggested under internal hemorrhoids and is given based on the scientific level (Banov et.al, 1985).

Grade 1: They have internal hemorrhoids that do not prolapsed.
Grade 2: They get internal hemorrhoids during defecation, but these resolve on their own.
Grade 3: They acquire internal hemorrhoids when they defecate, which usually require annual decrease
Grade 4: Internal hemorrhoids frequently prolapsed and become imprisoned.

**Causes**

Healthy people can get hemorrhoids; in fact, the uterus contains hemorrhoidal columns. The veins surrounding the anus have a propensity to expand or protrude when under pressure.

Hemorroid growth can occur for a variety of reasons, including obesity, constipation, diarrhea for extended periods of time, anal sex, low-fiber diets, frequent heavy lifting, etc (Mounsey et.al, 2011).

**Diagnosis**

**Patient History:** Hemorrhoids are frequently assumed to be the cause of anorectal discomfort by patients (Sardinha and Corman, 2002). Results pertaining to the frequency of bowel movements, the presence or absence of constipation or diarrhea, the duration of time spent on the commode, and the need for manual hemorrhoid reduction following bowel movements (Kann and Whitlow, 2004). Anoscopy, flexible sigmoidoscopy, or colonoscopy should be carried out based on the patient's age, medical history, co-morbidity risk, existence of alarm symptoms, and digital rectal examination.

**Physical examination:** A visual inspection is necessary to observe the external abnormalities in perianal area. Being more comfortable and generally less scary for patients than the prone position, the left lateral position is usually recommended. the detection of skin rashes, external hemorrhoids or tags, fistulae, abscesses, neoplasms, prolapse, condylomata, hypertrophic papillae, or any combination of these (Guttenplan and Ganz,2011).

**Anoscopy:** This method works best for looking at the anal canal and the farthest portion of the rectum. Anoscopes come in a variety of forms, including slotted and non-slotted varieties. Unlike non-slotted anoscopes, which lack such a cutout, slotted anoscopes have a cutout from the wall that permits the tissue in question to protrude into the slot, enhancing visibility. In a way that is not achievable with a flexible endoscope, both provide the chance to view the anus and distal rectum (Di Palma, 2011).

**Flexible endoscopy or Colonoscopy:** A sigmoidoscope, which is a thin, flexible tube, is used to execute it.
The tube has a camera and a little light. The tube is inserted into the anus and then carefully passed into the rectum and into the colon's lower section.
By forcing air into the colon through the tube, the colon somewhat swells to increase visibility (Di Palma, 2011).

**Treatments**

The medical management of hemorrhoid symptoms those are acute rather than the underlying cause of the condition. Flavonoids are used to treat hemorrhoids because they are known to increase vascular tone, decrease venous capacity, and decrease capillary permeability help promote the anti-inflammatory and lymphatic drainage effects (Lohsiriwat, 2015).

**Conservative medical treatment:**

The cornerstones of conservative medical treatment for hemorrhoids disorders are food and lifestyle changes. A diet that reduces the shearing effect of passing hard stool should involve increasing the intake of fiber and avoiding harmful foods. Topical medications, such as 0.2% glyceryl trinitrate are ineffective in treating grade 1 and grade 2 hemorrhoids with high anal canal pressure, and they are associated with a 43% headache rate in patients (Rivadeneira et.al, 2011).

**Surgical official based procedure**

**Pre-operative Preparation:**

These phase involves in hemorrhoids refers to the period before surgical procedure to treat hemorrhoids

**Intra-operative Phase:**

It refers to actual surgical procedure performed to address hemorrhoids. It involves the surgical removal of treatment of hemorrhoidal tissues.

**Phase Post-operative**

This phase involves in recovery and healing during which patients may experience pain, swelling and discomfort. It refers to the period of after surgical invention for hemorrhoids treatments.

**Post-operative Care**

These types of post –operative care that the patients need depends on the types of surgery as well as the patients history .It depends upon pain management and wound care.

**Non surgical official based procedure**

The most popular techniques for treating internal hemorrhoids include rubber band ligation, sclerotherapy, and infrared coagulation.

**Rubber band Ligation**

Rubber band ligation (Figure 2) to operate devices like anoscopes and ligatures. The internal hemorrhoids should prevent innervations of the surrounding tissue and the ligation's potential to cause ischemia, necrosis, and lumps that separate 5-7 days after the ligation (Cleator, 2005).

**Sclerotherapy**

In the sclerotherapy the affected area is not necessary, and the anticoagulants are visible. The sclerosant causes fibrosis, anal canal fixation, and the destruction of unnecessary hemorrhoidal tissue. Although the injection causes little to no pain, it might not be as effective as a rubber band (Bullock, 1997).

**Infrared coagulation**

The infrared coagulation techniques employ heat or infrared light that coagulates into tissue and evaporates in the cell by shrinking the hemorrhoids. The hemorrhoid base is applied through anoscope and contact, and the time between 1.0 and 1.5 depends on the patient's preference and any active hemorrhoids like thrombosis. They make tiny internal hemorrhoids that are bleeding harden and shrivel. Coagulation is uncomfortable and has few negative effects (Neiger, 1979).

**Management**

The management of hemorrhoids is based on their classification. The first treatment involves drinking a lot of water and eating a high-fiber diet, which helps the patient soften their faces and avoid straining during defection. The second treatment involves retraining patients to sit on the toilet safely. The third treatment involves using local and systemic treatments to control inflammation, treat vascular permeability, and enhance muscle contraction (Lalisang, 2016).

**Plant description :** (Gadekar et.al, 2023, Upadhyaya and Sathish, 2017, Sultana et.al, 2022) *Euphorbia neriifolia* is elegant and impulsive in gardens (Figure 3).The trunk and early branches are grayish and tubular, while them edium branches are free-shy and gently twisted.The branches range in length from 2-4meters. Succulent, deciduous leaves with 6–12 breadth,narrowing endson the branches of the plants. The tree has a cactus-like appearance. The green-colored stem of *E.neriifolia* has a cylindrical shape. It is made up of big, spherical branches. Sharp stipular thorns, a spiral ridge part,and a white-colored reticulate mass comprise its composition. The younger branchlets are verticulate, with whorls without articulation, mushy, with a thickness of 8 to 30 mm. Leafless and spine shield arranged in five different rows at about different angles.

The spines range in tint from brownish brown to black and are only 1-4 mm long. The setubercles are low, conical, and spirally organized; they are 2-3 cm apart and 2-5 mm tall. The oblong leaves of *E.neriifolia* measure (5-)10-18(-30) cm in length by (1.5-)3-4(-7.5) cm at the tip of the branches. The leaves are newly organized, oblong, hairless, and alternately formed. The majority of the year, with the exception of the monsoon, is leafless for the *E.neriifolia*. They are deciduous during the period of vegetation, but they fall in the late summer.

The same branch of laterally oriented axils contains both the male and female flowers. The upper lips display from three to seven floral panicles. These are reddish, flattened-globose, 1.5–2 mm x 4-5 mm, stiff, forked peduncles that are prominent in clusters of trees. While the lateral peduncles measure 6-7 mm, the central peduncle is subsessile. An inflorescence, or cluster of blooms, is a single female flower and several male flowers on the same limb of the plant. In essence, male flowers are numerous with linear bracts, but female flowers are rarely formed. December through May is when fruits and flowers are accessible. Smooth, with a diameter of 10–12 mm and a tiny notch, are the fruits (capsules).Moreover, they have three lobed or chambers. They emerge only in February and March and in varying climates. The substance called latex, found in the cells or capillaries that comprise the laticiferous system and having a milky sap-like consistency, usually results in tissue injury.

**Phytoconstituents** (Murali et.al, 2010)**:**
*Euphorbia neriifolia's* latex contains a variety of substances, including resin, neriifoline, euphol, euphorbon, and neriifoliol. The constituents yield various triterpenes, including ß-amyrin, taraxerol, nerifolione, cycloartenol, euphol, and euphorbiol. *Euphorbia neriifolia* glut-5(10)-en-1-one's leaves and stems were used to isolate an ovel triterpene. Research on a leaf extract containing ethanol revealed a novel flavonoid: 2- (3, 5-dihydroxy-6,7-dimethoxychromen-4-one)-3,4-dihydroxy-5-methoxy-phenyl. The hydroalcoholic extract of *E. neriifolia* contained triterpenoidal saponins, alkaloids, tannins, sucrose, and flavonoids, according to preliminary phytochemical investigation. Antiquorin has been isolated from a fresh *E. neriifolia* root ethanolic extract.

**Pharmacological aspects on hemorrhoids**
In accordance with OECD 423 criteria, Gulsheen et al. conducted acute toxicity experiments on mice using pet ether, chloroform, ethanol, and water extracts of *E. neriifolia.* When administered at a level of 2000 mg/kg, none of the extracts showed any symptoms of acute toxicity or fatality. (Gulsheen et.al, 2019) According to Kirtikar et al., "Kshaarasootra" is an Indian treatment that treats anal fistula by using E. neriifolia as one of its active constituents. (Kirtikar et.al, 1918) "Kshaarasootra" required a longer recuperation period (4 weeks as opposed to 2 weeks) but had a better prognosis in the long run. The results of the study showed that "Kshaarasootra" provided safe, ambulatory, and successful treatment for patients with fistula-in-ano. When "stannadi-vrana" is added and "shigru guggulu" (two tablets taken every day) is given orally during the course of treatment, "Kshaarasootra" shows great results in treating chronic nonhealing milk fistulae (Pawar et.al, 2023, Sultana et.al, 2022). Applying *E. neriifolia* leaves to piles relieves itching, discomfort, and edema (Khare, 2008). Parts of *E. neriifolia* are used in Madhusnuhi rasayana, an ayurvedic medication, to treat piles, according to Throat et al. and Prasanth et al. (Thorat and Bolli, 2017) and (Prasanth et.al, 2017) Bigonya et al. reported that *E. neriifolia* leaf extracts are useful in the treatment of hemorrhoids (Bigoniya and Rana, 2008). Oudhia et al., Ahmed et al., Chaudhary et al. and Sharma et al. proposed that application of lukewarm *E. neriifolia* leaves reduces itching pain, swelling and bleeding in piles. (Oudhia et. al, 2003) ( Ahmed et. al, 2011) ( Chaudhary et.al, 2021) and ( Sharma et. al, 2011).

## CONCLUSION

The different types of hemorrhoids with the normal anorectal structure are shown in the Figure 1. While colonoscopy revealed only 78% of the lesions when the scope was withdrawn straight and only 54% when it was retroflexed, (Kelley et.al, 1986) reported that anoscopy detected 99% of the lesions in patients' anals. For patients with hemorrhoidal problems, nonsurgical methods are successful in 80%–99% of cases. For grade III hemorrhoids, surgical hemorrhiodectomy is a more successful treatment than radiation therapy (Figure 2). To avoid the surgery different extracts as well as latex of *E. neriifolia* is used to treat the hemorrhoids. Different plant parts were shown in Figure 3. The plant constituents are described in the phytoconstituent section. Some phytoconstituents that have been identified to have pharmacological effects include tannins, flavonoids, alkaloids, and triterpenoidal saponins. OECD guideline 423 states that extracts of *E. neriifolia* in pet ether, chloroform, ethanol, and water are safe. The usefulness of *E. neriifolia* against hemorrhoids as well as pain and bleeding associated with hemorrhoids also discussed. The surgical methods are very risky and painful for the treatment of hemorrhoids. In India, traditional medical systems

are frequently used. A sizable segment of the populace, primarily from rural areas, still relies on the old medical system. Medicinal plants are used in alternative medicine to cure illnesses that are resistant to conventional treatment. Sehund latex appears to have magical qualities and has been well studied. The plant *E. neriifolia* is a rich source of phytoconstituents that are therapeutically effective for the treatment of hemorrhoids, and this provides a wider zone of interest for the discovery of new molecules for drugs, as was evident from the thorough study and investigation of the literature that is currently available on the subject. The chronic toxicity of *E. neriifolia* has to be investigated, even if the acute toxicity has already been investigated. Future research on these topics should aid in the creation of novel, potent hemorrhoid medications.

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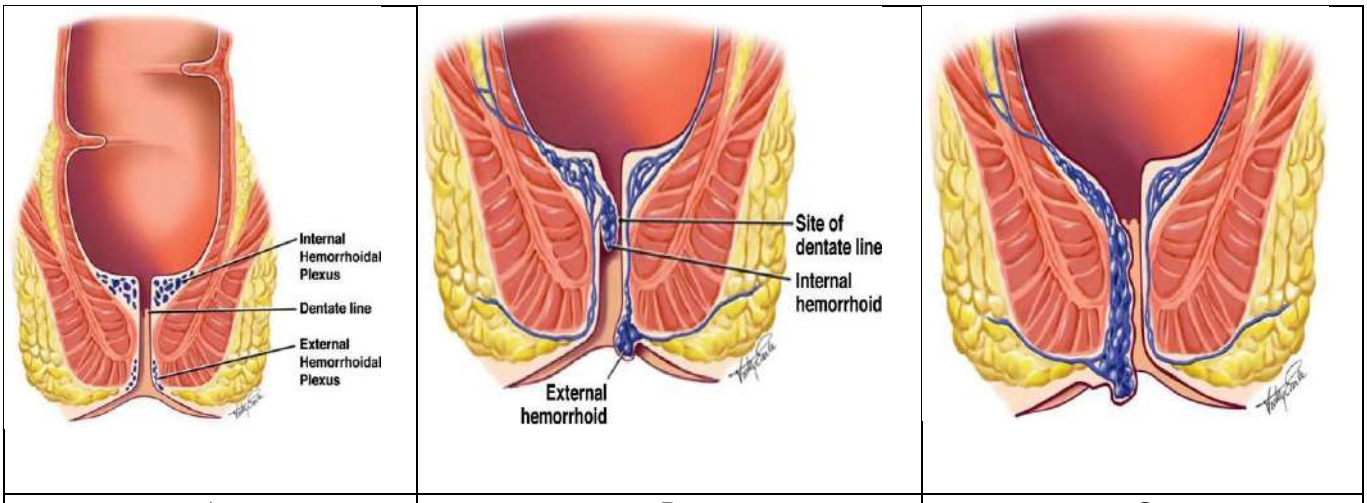


| A | B | C |
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**Figure 1. Illustration of Normal anorectum (A); Illustration of Internal and External hemorrhoid(B); Illustration of Mixed hemorrhoid (C) (Ganz, 2013).**

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| **Figure 2. Rubber band ligation of internal hemorrhoids (Ganz, 2013)** | **Figure 3. *Euphorbia neriifolia* plant with fresh leaves** |

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**RESEARCH ARTICLE**

# Balancing your Biome for Better Mental Health: Gut Microbiota and Depression

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**ABSTRACT**

Recent research highlights a significant link between the gut microbiome and mental health, particularly regarding depression. Termed the 'second brain,' the gut is inhabited by trillions of microorganisms that regulate neurotransmitters and produce compounds impacting emotional well-being. This complex interplay between gut microbes and the brain influences mood, anxiety, and depression. Studies suggest probiotics and dietary changes as potential therapeutic strategies for managing depression by fostering a healthy gut microbiome. This emerging field emphasizes the gut microbiome's crucial role in mental health, offering new avenues for treating depression and enhancing overall well-being.

**Keywords:** Gut microbiome, Depression, Gut brain axis, Probiotics, Prebiotics, Diet.

## INTRODUCTION

### Gut Microbiome Definition

The gut microbiome, an intricate ecosystem within the gastrointestinal tracts of humans and animals, is composed of diverse microorganisms, including bacteria, viruses, fungi, and others, playing crucial roles in the host's physiological processes [1]. This ecosystem, also known as the gut microbiota when referring to the microbial

community itself, is predominantly found within the digestive tracts of humans, animals, and insects, highlighting its universal presence. After birth, the digestive tract quickly becomes colonized by a variety of microorganisms such as bacteria, viruses, and complex-celled eukaryotes. This colonization is crucial for the gut microbiome's initial development and function [2]. The gut microbiota boasts over 1500 species across more than 50 phyla, a classification of organisms based on common traits. Notably, Bacteroidetes, Firmicutes, and Proteobacteria are dominant phyla, comprising up to 90% of the microbial population [3].

The composition and function of the gut microbiota are influenced by factors like genetics, diet, age, birth conditions, and antibiotic exposure. These elements shape the gut microbiome's dynamic and adaptable nature [4]. The gut microbiota's roles are fundamental to the host's health. It protects against harmful pathogens, produces antimicrobial substances, bolsters the immune system, aids in metabolism, affects insulin sensitivity, and communicates with the brain through the gut-brain axis—a two-way connection between the nervous system and the gastrointestinal tract. Thus, the gut microbiota is essential for maintaining gut health and overall well-being [5-8].

**Overview of Depression**

Recent advancements in understanding depression, a widespread and severe mental disorder, have been notable [9]. These improvements stem from reduced societal stigma, enhanced treatments, and primary care physicians' increased engagement with this condition. Depression takes multiple forms, such as major depressive disorder (MDD) and intermittent episodes, impacting diverse groups including pregnant women, adolescents, and the elderly [10]. Understanding depression involves exploring its genetic and environmental roots [11]. Major depressive disorder, affecting an estimated 350 million people globally, significantly contributes to the global disease burden [12]. Studies reveal that depression involves complex interactions between neuroendocrine and neuroimmune pathways, which link inflammation to cognitive functions [13]. The gut-brain connection has emerged as a key area in depression research. This connection underscores the importance of a healthy gut microbiome for mental well-being. It entails a bidirectional communication system between the gut and brain, influencing emotions, cognition, and motivation, while balancing gastrointestinal health. This system integrates the central nervous system (CNS), autonomic nervous system (ANS), enteric nervous system (ENS), and the hypothalamic-pituitary-adrenal (HPA) axis through neuro-immuno-endocrine mediators [14].

The gut microbiota, essential in this interaction, has been linked to mental health implications [11]. While research has revealed the gut microbiome's impact on depression, it also highlights the need for more comprehensive study designs [14]. The role of probiotics and the influence of gut microbiota on psychiatric conditions are areas requiring further investigation [15]. Understanding the relationship between gastrointestinal microflora changes and depression is crucial, offering potential new treatment insights [16]. Ongoing research continues to explore environmental factors in major depressive disorder, furthering our understanding of its complex nature [17].

**Research Insights and Future Directions**

Research on the microbiome gut-brain axis has illuminated the role of enteric microbiota in mental health, especially concerning conditions like depression [11]. However, there are limitations in current research methodologies regarding the gut microbiome's impact on depression [14]. Probiotic interventions and the role of gut microbiota in psychiatric conditions have been explored, but further study is necessary for clearer understanding [15]. Unravelling how changes in gastrointestinal microflora relate to depression is key to deciphering the gut-brain axis [16]. Although there has been progress in linking gut microbiota to depression, investigating environmental factors in major depressive disorder remains a critical area of ongoing research [17].

**UNDERSTANDING THE GUT-BRAIN AXIS**

**Vagus Nerve - Role in Connecting Gut and Brain - Influence on Mood**

The Vagus Nerve (VN) is a central component of the body's nervous system, linking various visceral organs to the brain, including the gastrointestinal tract, respiratory system, and cardiovascular system. Its branches, especially the hepatic and celiac branches, connect to afferent neurons in the gut's nodose ganglia, converging at the brainstem's

nucleus tractus solitarius (NTS) [18]. From the dorsal motor nucleus of the vagus nerve (DMNV), efferent fibres extend, closely associated with the NTS, highlighting the VN's role as a vital communication pathway between the gut and brain [18]. Vagal afferents (VA) detect diverse signals from the gut, such as tension, stretch, and various biochemical markers, including hormones and gut bacteria by-products [19]. This sensory input informs the brain about the gastrointestinal system's status, influencing physiological processes like gastrointestinal motility, acid secretion, food intake, and satiety. The NTS and DMNV form an autonomic brainstem loop, with projections extending to the central nervous system (CNS), modulating neuroendocrine responses, emotions, cognition, and behaviours [19]. This underscores the VN's critical role in gut-brain communication.

Moreover, the VN indirectly conveys gut-related insights through interactions with enteroendocrine cells (EECs). These cells release mediators like serotonin and peptide YY, impacting food intake and autonomic reflexes controlling gut functions such as motility and inflammation [20]. Neuropods, a subset of EECs, transmit signals to vagal neurons via neurotransmitters like glutamate [21]. Complementing the VN's role, the Enteric Nervous System (ENS) acts as the gut's local control centre, regulating functions such as peristalsis and blood flow. Its connections to the CNS, facilitated by the VN and sympathetic nerves, highlight the significance of neural communication in gut physiology [22]. Additionally, the influence of microbiota metabolites on ENS activity and gut motility, particularly evident in rodent models, reveals the growing understanding of gut microbiota's role in neural pathways [23]. The VN serves as an essential conduit in these interactions, further emphasizing its role in maintaining gastrointestinal homeostasis and linking gut health with overall well-being.

**Immune System - Inflammation and its Relation to Depression**

Recent research has illuminated the complex relationship between the gut microbiota and the brain, highlighting the pivotal role of the endocrine and immune systems in this interaction [24]. Central to this connection is the hypothalamic-pituitary-adrenal (HPA) axis, a key component of the endocrine system, which is significantly influenced by the gut microbiota composition [25]. Studies in germ-free mice have demonstrated that stress affects the gut microbiota, altering HPA axis hormones, increasing corticotropin-releasing factor (CRF) levels, and reducing glucocorticoid receptor (GR) expression. These changes exemplify the profound impact of gut microbes on our stress response system.

Stress not only disrupts the gut microbiota balance but also increases gut permeability. This effect is mediated by the growth of certain gram-negative bacteria and heightened CRF levels, which contribute to gut dysfunction. The increased permeability allows bacteria and their metabolites to enter the bloodstream, leading to localized inflammation. This inflammation is characterized by a rise in pro-inflammatory cytokines, further activating the HPA axis and increasing cortisol secretion [26]. Another key factor in this process is lipopolysaccharide (LPS), found in the outer membrane of Gram-negative bacteria. LPS activates toll-like receptors on macrophages, triggering the release of pro-inflammatory cytokines [24]. This response affects not only the immune system but also the HPA axis, influencing the brain and leading to symptoms known as sickness behaviour. The gut microbiota's composition and diversity, including phyla like Bacteroidetes and Firmicutes, play a significant role in this gut-brain-immune system interplay [24]. These microbial populations are shaped by factors such as maternal health and early childhood development. Imbalances in these microbial communities, known as dysbiosis, are linked to a range of disorders, including gastrointestinal issues like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), as well as neuropsychiatric conditions such as depression, anxiety, autism, and anorexia. Hopefully, interventions like probiotics and dietary modifications have shown potential in restoring gut microbial balance, offering relief from symptoms associated with these disorders [24]. These findings underscore the potential of targeting the gut microbiota in treating various health conditions.

**Factors affecting microbiome gut brain axis**

**Genetics and Epigenetics**

The intricate relationship between genetics and epigenetics is crucial in understanding how host genetics affect microbiota composition, particularly in brain health, a field that remains underexplored [30]. Microbiota plays a key

role here, as shown by strong correlations in taxa abundance among monozygotic twins, highlighting the heritability of certain microbiota families like Christensenellaceae. Beyond genetic influence, microbiota is increasingly recognized as an epigenetic entity, influencing the host genome through gene-environment interactions [31]. In the gut, HDAC3's role in epithelial cells is vital for maintaining host-commensal signalling. Inhibiting HDAC activity can reverse stress-induced visceral hypersensitivity and anxiety, conditions dependent on the microbiota-gut-brain axis [32]. Furthermore, micrornas (mirnas), essential in gene expression regulation, are also influenced by the gut microbiota, affecting mirna expression in brain regions [32, 33]. Significantly, changes in mirna expression patterns in the amygdala and prefrontal cortex, linked to gut microbiota, have been observed in germ-free mice. These changes can be reversed with microbiota recolonization [33]. In conclusion, the combined impact of genetics, epigenetics, and microbiota is pivotal in brain health, warranting further investigation in this emerging field.

**Diet**

Diet profoundly shapes gut microbiota composition, which in turn significantly influences brain function and behaviour. Gut microbiota, the community of microorganisms in our intestines, is pivotal for health. Rapid changes in this composition can occur within 24 hours of dietary changes, affecting mood even in individuals without mood disorders [34,35]. This highlights the intricate connection between what we eat and our mental well-being. Dietary patterns, especially the contrast between animal-based and plant-based diets, markedly affect microbiota diversity. Lifestyle shifts and food preferences further modify this relationship. Notably, dietary iron plays a crucial role in managing insulin resistance and infection susceptibility, emphasizing the importance of diet in health maintenance [36-42]. Different diets lead to unique microbiota profiles with varied health outcomes. The Western diet, rich in sugar, salt, and fat, mirrors the gut microbiota of obesity and can quickly modify gut bacteria, increasing inflammatory Proteobacteria. Conversely, the Mediterranean diet, abundant in fruits, vegetables, and whole grains, fosters a healthier microbiota, linked to reduced depression risk and lower levels of harmful bacteria [43-48].

The ketogenic diet, known for high fat and low carbohydrates, benefits several neurological conditions. It alters the gut microbiota by boosting beneficial bacteria like Akkermansia and Parabacteroides, impacting the gut-brain communication axis [51]. Carbohydrates play a crucial role in shaping gut microbiota. Digestible carbohydrates such as glucose can increase beneficial bacteria like Bifidobacterium, while indigestible carbohydrates, or prebiotics, promote overall gut health by nurturing beneficial microorganisms [52]. Proteins, from both plant and animal sources, significantly influence gut microbiota. Plant-based proteins correlate with greater diversity, whereas animal-based proteins affect specific bacterial populations. This underscores the impact of protein type on gut microbiota composition [52,53]. Various fats exert unique influences on gut microbiota. Saturated and trans fats, common in the Western diet, may reduce beneficial bacteria. In contrast, omega-3 polyunsaturated fatty acids enhance gut health by increasing beneficial microbes and diversity, demonstrating the critical role of fat quality in diet [54-56].

**Environment**

Environmental factors significantly influence health outcomes and gut microbiota across cultures. One key mechanism is the metabolism of environmental chemicals by gut microbiota, which affects its composition and, in turn, may impact brain function and behaviour via the microbiota-gut-brain axis [57]. For instance, Bisphenol A (BPA), commonly found in plastic containers, exemplifies this impact. As an endocrine-disrupting chemical, BPA, when consumed, reduces gut microbiota diversity, notably increasing Proteobacteria and Helicobacteraceae and decreasing Clostridia [58]. These changes underscore the delicate balance within our gut ecosystem. Similarly, heavy metals like cadmium, mercury, arsenic, and lead pose serious health risks, causing carcinogenicity, immune disruption, nucleic acid damage, and oxidative stress [59]. Exposure to these metals alters gut microbiota, evidenced by increased Lactobacillaceae and Erysipelotrichaceae and decreased Lachnospiraceae in animals. This shift in microbiota composition, particularly the reduction of Lachnospiraceae linked to depression, suggests a potential microbiota-gut-brain connection influenced by environmental toxins.

Furthermore, the widespread use of antibiotics, leading to their accumulation in natural environments like lakes, rivers, and agricultural soil [60-62], represents another environmental challenge. This increase in environmental antibiotics threatens not just individual health but also global ecological balance [63].

**Exercise**

Regular exercise significantly improves brain health, as seen in rodents where it enhances neurogenesis and counters aging-related hippocampal decline [64]. It also positively influences gut health, enhancing microbiota diversity. This leads to reduced stress, better immunity, and improved energy regulation in the gut microbiota. Despite these insights, the interplay between exercise and gut microbiota, especially how the latter affects exercise performance, is still not well-understood [65]. Animal studies reveal that exercise alters the microbiota to prevent diet-induced obesity and improve glucose tolerance. In humans, increased physical activity is associated with greater diversity in gut microbiota, notably in the Firmicutes phylum [66]. The cessation of regular exercise can quickly result in mood deterioration and fatigue. This is possibly linked to the gut microbiota's influence on tryptophan metabolism and kynurenine levels, which are related to depression. Exercise's benefits are thought to involve the kynurenine pathway and the activation of PGC-1$\alpha$1 in muscles, which may bolster resistance to chronic stress and CNS inflammation.

**Medications**

Medications profoundly affect the gut microbiota, which encompasses the diverse microorganisms in our digestive system. This impact extends beyond antibiotics to non-antibiotic drugs, influencing behaviour and how drugs work in the body [67,68]. Common medications, including antibiotics, laxatives, IBD treatments, female hormones, benzodiazepines, antidepressants, and antihistamines, significantly alter the gut's microbial composition [69]. Advanced sequencing studies have linked 44 drug categories to changes in the gut microbiota. This relationship spans various drugs, including proton pump inhibitors (ppis), metformin, statins, and laxatives, each altering the microbiota in distinct ways [70]. Polypharmacy, the use of multiple medications simultaneously, correlates with reduced microbial diversity in the gut. Specific microbes are especially affected by proton pump inhibitors, antidepressants, and antipsychotics. Meta transcriptomic research, which analyses gene expression in microbes, shows that short-term exposure to non-antibiotic drugs alters genes involved in drug processing. This suggests these drugs can also affect microbial function and potentially influence brain function [71-73]. Antidepressants, such as SSRI'sand TCA's, demonstrate antimicrobial properties against certain bacteria, influencing gut microbiota composition [74,75]. Moreover, phenothiazines, a group of antipsychotic drugs, offer dual benefits: combating bacterial infections and modifying the gut microbiota. This is particularly relevant to the microbiota-gut-brain axis, the complex interaction network between the gut microbiota and the brain [76-79].

**Stress**

Stress disrupts an organism's equilibrium, initiating cortisol release via the HPA axis to prepare for threat response [80]. Chronic stress can lead to HPA axis dysregulation, increasing the risk of health issues like anxiety and depression, which impose significant economic impacts. Research spanning over four decades and encompassing various species, including rodents and non-human primates, consistently shows that stress alters gut microbiota composition [81-86]. This alteration extends to maternal stress during pregnancy, which shapes the microbiota and HPA axis reactivity in offspring.Notably, transferring vaginal microbiota from stressed dams to nonstressed offspring can modify adult stress responses in these offspring [87]. Human studies corroborate these findings, indicating that infants born to mothers with high cumulative stress during pregnancy exhibit microbial composition changes. The microbiota-gut-brain axis is influenced by daily factors, from food cravings to inherited traits and birth delivery methods, highlighting the need for further research into how microbiota influences behaviour and disease development.

**Fear**

The regulation of fear, a complex cognitive process, is significantly influenced by both the stress response and the gut-brain axis, with notable implications for clinical anxiety. Key brain regions such as the amygdala, hippocampus,

and prefrontal cortex (PFC) are integral to this process. Recent studies highlight the microbiota's role in modulating these brain regions' functions [88-91]. Functional MRI (fmri) studies in humans reveal fascinating links between gut microbiota and brain activity during fear stimulus processing [92,93]. For instance, healthy women who consumed a fermented milk product with probiotics showed reduced brain responses to negatively emotional faces, particularly in areas involving the PFC.

Parallel research in animal models sheds light on the microbiota's role in fear response, especially in memory and extinction. Experiments with Germ-Free (GF) mice reveal that lacking microbiota impairs short-term fear recall, leading to diminished fear responses and altered amygdala gene expression (94). Additionally, studies on humans and rodents show that acute antibiotic exposure disrupts fear memory and enhances extinction capabilities [95]. Probiotic examinations reveal strain-specific impacts on fear learning and memory. For example, B. Longum 1714 boosts fear learning and short-term memory, while L. Rhamnosus (JB-1) decelerates extinction. Certain probiotic strains, like L. Rhamnosus R0011 and L. Helveticus R0052, may mitigate early-life stress effects on fear behaviour and related neural networks during development [96,97].

**Circadian rhythm**

Circadian rhythms, fundamental 24-hour cycles in bodily functions, are disrupted by phenomena like jet lag and shift work, leading to metabolic and psychiatric disorders [98]. Research is uncovering the complex relationship between these rhythms and the microbiome, especially in obesity, cardiovascular disease (CVD), diabetes, and neurodegenerative disorders. This work sheds light on the microbiota-gut-brain axis, a key player in these conditions [99-101]. Importantly, the microbiota can modulate the circadian clock, influencing both peripheral and central timing systems. Circadian disruptions disturb the balance of intestinal microbiota, but timed feeding shows promise in countering these effects (100). Studies using Germ-free (GF) mice highlight the microbiota's role in regulating liver clock oscillations, crucial for maintaining liver function and metabolic gene expression [102]. Shift work and jet lag can lead to an arrhythmic microbiome, marked by significant changes in its composition. These changes, linked to metabolic imbalances, can be transferred to GF mice through faecal microbiota transplantation (FMT). As such, targeting the microbiota could be an effective strategy for managing conditions associated with circadian rhythm disturbances, particularly when combined with unhealthy diets. This interplay between circadian rhythms, microbiota, and brain health is of significant physiological and potential clinical importance [101,103].

**Food intake**

Microbiota's Role in Regulating Food Intake: The gut microbiota plays a pivotal role in controlling food intake through hormones that regulate appetite [104, 105]. Disturbances in this microbiota are linked to conditions like anorexia nervosa and obesity [106, 107]. Notably, bariatric surgeries, including Roux-en-Y gastric bypass, effectively treat obesity by altering gut microbes. This suggests that these microbes may evolve to manipulate eating behaviour, possibly by inducing cravings or dysphoria until the host consumes nutrients beneficial to them. Gut microbes produce proteins, like caseinolytic protease B (clpb), that mimic appetite-regulating peptides, thereby influencing food intake [109]. This mimicry potentially disrupts hormone degradation. The microbiota may also affect nutrient sensing and taste perception, especially in conditions like obesity and anorexia nervosa where taste is often altered [110, 111]. The immune system's role in maintaining taste receptors further complicates this interaction.

**Social behaviour**

Exploring Microbiota's Role in Animal Social Behaviour: Studies reveal a significant link between the microbiota and social behaviour in animals. Notably, germ-free (GF) mice, which are devoid of microbiota, display reduced social interactions and face challenges in recognizing new individuals of their species, highlighting the microbiota's critical role [112-115]. Furthermore, antibiotic treatments in rodents that diminish microbial diversity have been linked to deteriorated social behaviours [116]. The mechanisms by which microbiota influence social behaviour, though not fully unravelled, involve complex biological interactions. Key findings suggest that components from gut bacteria, such as peptidoglycan, can influence brain regions like the amygdala, essential for social interactions [115, 117]. Altering the microbiota through diet or probiotics also shows promising results in modulating social behaviour. The

administration of *L. Reuteri* to mice enhances hormone levels associated with sociability, and the use of probiotics like VSL#3 alleviates social withdrawal symptoms while decreasing inflammation markers in the blood [112].

## THERAPEUTIC APPROACHES

### Prebiotics - Efficacy in Treating Gut Microbiome-Related Depression

Prebiotics, increasingly recognized in mental health, offer an innovative approach to managing cognitive and mood disorders through gut health enhancement. Unlike probiotics, they promote beneficial bacterial growth, notably Lactobacillus species, crucial for maintaining the intestinal barrier and reducing pro-inflammatory stimuli, thereby addressing dysbiosis and associated inflammation [120]. As immune modulators, prebiotics decrease pro-inflammatory cytokines and oxidative stress markers. They also produce short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate during fermentation by gut microbiota, playing a vital role in immune regulation and potentially mitigating inflammation in central nervous system diseases. [121] In mood disorders such as major depressive disorder (MDD) and anxiety, prebiotics like fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) have shown promise. Animal studies indicate that these prebiotics can lessen depressive behaviours and exert anxiolytic effects, correlating with reduced plasma corticosterone and altered gene expression in brain regions involved in key neurotransmitters [122]. Moreover, they alleviate symptoms like anhedonia in stressed animals, increasing beneficial gut bacteria like Bifidobacterium and Lactobacillus.

Despite these findings, the application of prebiotics in clinical settings for mood disorders remains challenging. Clinical studies present mixed results; for instance, Bimuno-galacto-oligosaccharides (B-GOS) were found to reduce salivary cortisol and affect emotional processing, whereas the impacts of other prebiotics like FOS were less evident. Some trials reported no significant mood or inflammation improvement compared to probiotics [123]. This inconsistency highlights the necessity for further research to fully understand prebiotics' therapeutic potential in treating depression and anxiety [124].

### Probiotics - Efficacy in Treating Gut Microbiome-Related Depression

In the realm of precision psychiatry, an approach that personalizes treatment based on individual genetic, environmental, and lifestyle factors, probiotics—specifically Lactobacillus and Bifidobacterium strains—have shown promise [125-132]. These live microorganisms are gaining attention for their potential to correct imbalances in the gut microbiota, a condition often linked with depression. Such imbalances are marked by a lack of microbial diversity and the depletion of specific bacterial genera. This understanding underscores the role of probiotics in reinstating gut equilibrium, thereby contributing to improved mental health outcomes. Central to this concept is the microbiota-gut-brain axis (MGBA), a bidirectional communication system that highlights the impact of probiotics on neurobehavioral health. Through intricate interactions involving the nervous and endocrine systems, probiotics exert a positive influence on this axis [133-135]. Their ability to effectively reach and act within the gastrointestinal tract makes them viable candidates for targeted psychiatric interventions, aligning well with the principles of precision psychiatry.

### Dietary Changes - Impact on Depression

Diet significantly influences gut bacteria, surpassing genetic factors. Beneficial elements like plant proteins, unsaturated fats, and dietary fibre promote a healthy gut microbiota. Conversely, diets high in animal proteins, saturated fats, and simple sugars correlate with less favourable gut bacteria [136]. The Western diet, rich in saturated fats, processed foods, and refined sugars, leads to a gut microbiota profile characterized by reduced diversity and increased gut permeability. Such changes are linked to metabolic syndrome and chronic diseases [137]. Dietary fibre is crucial for immune health, aiding in producing short-chain fatty acids (SCFAs) by gut bacteria. This process is essential for maintaining robust immune function [138]. Digestive disorders, which affect a significant American population segment, pose a $100 billion annual economic impact. Medications like proton pump inhibitors (PPIs), such as omeprazole, can disrupt gut microbiota diversity, increasing gastrointestinal infection risks. This impact is more significant than that of antibiotics [139].

Equilibrium in gut bacteria is vital for health, preventing dominance by any single species. Imbalances can lead to irregular eating habits [140]. Gut bacteria influence food choices by mimicking or interfering with human appetite-regulating hormones, affecting reward pathways and potentially taste receptors [141]. They also impact mood through neurotransmitters like serotonin, influencing eating behaviours [142]. Dietary choices affect stress and depression. Sugar consumption may reduce stress responses, suggesting a potential preference for sweets under stress [143,144].

## CONCLUSION

In conclusion, the gut microbiome is a dynamic and intricate ecosystem with significant impacts on human health. Understanding its relationship with the gut-brain axis, neurotransmitters, and dietary factors opens new avenues for treating mental health issues, particularly depression. Continued research into probiotics, prebiotics, and dietary changes holds promise for unlocking their full therapeutic potential.

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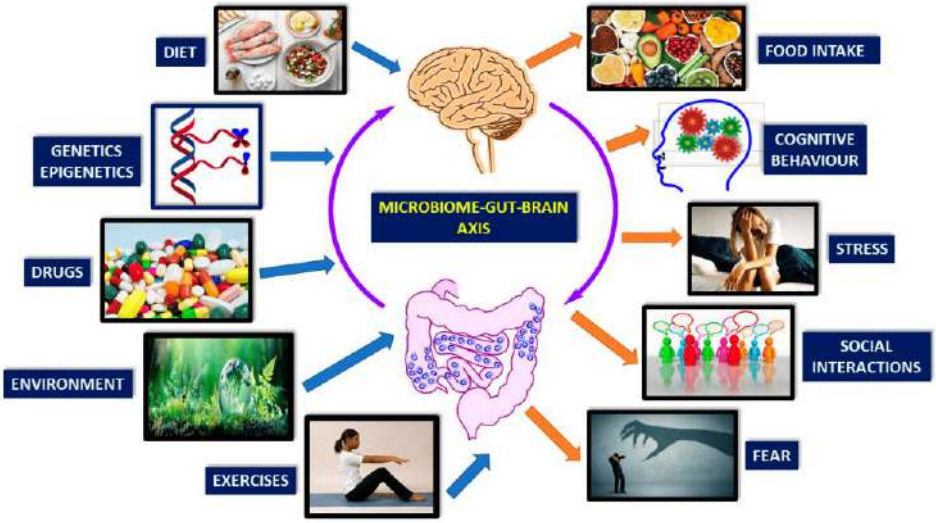


**Figure 1: Factors affecting microbiome gut brain axis [29]**

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**RESEARCH ARTICLE**

# M/D/1 Queuing Model with Picture Fuzzy Set

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**ABSTRACT**

This paper introduces the integration of Picture Fuzzy Sets (PFS) into the M/D/1 queuing model with an infinite queue capacity. To capture the uncertainties inherent in both arrival and service processes, we model the entry and utility rates using Single Valued Trapezoidal Picture Fuzzy Numbers (SVTPN). The DSW algorithm and the ($\propto$, $\beta$, $\gamma$) cut method are utilized to develop our proposed model. Our analysis employs a hierarchical approach, evaluating the system based on varying ($\propto$, $\beta$, $\gamma$) cuts. This multi-level approach effectively illustrates how membership values are represented and managed within the SVTPN framework. Through a detailed numerical example, we demonstrate the implementation and benefits of our fuzzy-based method in queuing system analysis. Future research will extend these findings to more complex and varied queuing scenarios and exploring the potential of picture fuzzy set theory in operational analysis.

**Keywords:** Picture Fuzzy Set,single valued trapezoidal picture fuzzy number,DSW algorithm, ($\propto, \beta, \gamma$) cut

## INTRODUCTION

Queuing theory has evolved since the early 1900's in three main phases. It was born with the work of A.K. Erlang, who published in 1917 the important formulas which now bear his name. Erlang's motivation was to develop tools for the analysis and design of telephone systems, an application that continues to the present day to motivate research in queueing theory. Renewed interest in queueing theory and its potential applications outside of telecommunications came with the codification of the field of operations research in the early 1950's. The use of

queueing theory as a tool for the performance evaluation of computer systems and components of computer systems is now well established, with the result that most undergraduate computer science majors receive at least some exposure to its models and methodology. The present trend combining computing and telecommunications promises an acceleration in the development of queueing theory and its importance to computer scientists and engineers.

Queuing models are fundamental tools in the study of systems where entities wait for service. They provide insights into system performance, guiding the design and optimization of various operational processes. Traditional queuing models, like the M/D/1 queue, rely on precise numerical inputs for arrival and service rates. However, real-world systems often exhibit uncertainties that challenge the precision of these parameters. To address these uncertainties, fuzzy set theory has been widely applied to queuing models, providing a more flexible approach to modeling and analysis. Fuzzy sets, introduced by Zadeh (1965) (22), allow for a gradual assessment of membership rather than a binary classification. This concept was further expanded by Atanassov (1986) (2) into intuitionistic fuzzy sets, which account for degrees of membership, non-membership, and hesitation. These advancements paved the way for more nuanced fuzzy models, such as the trapezoidal fuzzy numbers used by Sumathi and Antony Crispin Sweety (2019) (16) and the single valued neutrosophic sets discussed by Wang et al. (2010) (26). The integration of fuzzy sets into queuing models has been extensively studied. Li and Lee (1989) (8) explored the analysis of fuzzy queues, demonstrating how fuzzy logic can enhance the understanding of queuing dynamics. Similarly, Kalpana and Anusheela (2018) (4) analyzed a single-server non-preemptive fuzzy priority queue, showcasing the utility of fuzzy sets in prioritizing service. The work of Rashad and Mohamed (2021) (9) on neutrosophic theory in queuing models further illustrates the application of advanced fuzzy concepts to capture even more complex uncertainties.

Kumuthavalli and Sangeetha (2017) (7) introduced neutrosophic fuzzy sets to queuing theory, emphasizing their role in modeling ambiguous and conflicting information. This approach aligns with the developments in neutrosophic sets by Smarandache (2010)(13), (2020)(14), who explored their applications in various operational scenarios. The neutrosophic extension of fuzzy sets allows for a more comprehensive representation of uncertainty, which is particularly useful in the context of queuing models with variable parameters. In the realm of performance evaluation of fuzzy queuing systems, the DSW algorithm has been a prominent tool. Srinivasan (2014) (15) and Shanmugasundaram and Venkatesh (2015) (10) employed the DSW algorithm to analyze fuzzy queuing models, demonstrating its effectiveness in handling fuzzy data. The algorithm's ability to process fuzzy numbers into crisp values makes it a valuable asset in operational research and system analysis. A picture fuzzy set (PFS) is a mathematical technique in queuing theory that is used to analyse and characterize complicated systems with uncertainty. A PFS is made up of several fuzzy sets that work together to describe inaccurate or hazy information about a system, including how consumers or servers behave. When modelling and assessing the performance of queuing systems, where inaccurate data or presumptions could result in wrong results, this approach is helpful. Because PFS-based models take into account the uncertainty inherent in real-world systems, they can yield forecasts and judgements that are more accurate.

The picture fuzzy set, introduced by Bui Cong Cung (2014) (3), extends the intuitionistic fuzzy set by adding a fourth parameter, capturing the degree of neutrality. This additional parameter allows for a richer representation of uncertainty, making picture fuzzy sets a powerful tool for modeling complex systems. This extended model is particularly useful in situations where decisions or classifications cannot be strictly binary or even dualistic but require an intermediate or neutral state. For instance, in sentiment analysis of social media posts, picture fuzzy sets allow for a more nuanced understanding of public sentiment, capturing not just positive and negative sentiments but also neutral or ambivalent feelings (Thakkar et al., 2021) (17). Moreover, the picture fuzzy sets have found applications in medical diagnosis, where patients' conditions can be classified not just as positive or negative for a particular disease but can also consider indeterminate states or varying degrees of symptom severity (Thao et al., 2019). This (18) granularity improves decision-making processes by providing a richer set of information. The picture fuzzy set's application to queuing models offers a new dimension in analyzing systems with ambiguous and imprecise data. Building on these foundational works, our paper introduces the use of Picture Fuzzy Sets (PFS) in the M/D/1 queuing model with an infinite queue capacity. We represent both the arrival rate and service time using

Single Valued Trapezoidal Picture Fuzzy Numbers (SVTPN), following the approach of Varadharajan and Susmitha (2018) (21) who evaluated performance measures of priority queues with fuzzy parameters using the $\alpha$-cut method. The DSW algorithm and the ($\propto$, $\beta$, $\gamma$) cut method are utilized to compute the system's performance metrics, aligning with the methodologies proposed by Usha Prameela and Pavan Kumar (2019) (19) (20) in their study of fuzzy multi-server queuing models. Building on these concepts, Shi, Kosari, and Khan (2023) (12) introduce interval-valued picture fuzzy graphs, which combine the strengths of interval-valued fuzzy graphs and PFSs. This new model enhances the ability to manage and analyze data characterized by multiple degrees of membership, non-membership, and hesitancy. Our analysis is structured across primary, secondaryand tertiary levels, each corresponding to different ($\propto$, $\beta$, $\gamma$) cuts, to capture the varying degrees of uncertainty within the system. This multi-level approach provides a comprehensive view of the system's performance under different fuzzy scenarios. The practical implications of our model are demonstrated through a numerical example, highlighting the benefits of using picture fuzzy sets in queuing analysis. This approach not only captures traditional uncertainties but also accommodates the degrees of membership, non-membership, and hesitation inherent in real-world systems.

Future research will extend our findings to more advanced fuzzy set models, such as Type-II fuzzy sets and Pythagorean fuzzy sets, as explored by Kanufmann (1975) (5) and Smarandache (2020). These models can capture even higher levels of uncertainty and complexity, providing deeper insights into the dynamics of queuing systems. Our study contributes to the ongoing development of fuzzy set theory and its application in queuing models, offering a novel approach to understanding and managing uncertainty in operational processes. The purpose of this research is to look at a single server queuing model with fixed utility period that uses single valued picture fuzzy set (SVPS). The suppositions andcalculationsareexplainedinpartthree.Thefourthparthastheproposed queuing miniature. The fifth part addresses the present model's result strategy and sixth partcontains the conclusion and results.

**Basic definitions**

**Definition 2.1 [3].** A picture fuzzy set A on a universe X is an object in the form of
$A = \{x, (\mu_A(x), \eta_A(x), \gamma_A(x))\}$ ; (x € x}
Where $\mu_A$ (x) € [0.1] is called the degree of positive membership of x in A, $\eta_A(x)$ € [0,1] is called the degree of neutral membership of x in A and $\gamma_A(x)$ € [0,1] is called the degree of negative membership of x in A, and where $\mu_A$, $\eta_A$, and $\gamma_A$ satisfy the following condition:
$(\forall x \in X)\ (\mu_A(x) + \eta_A(x) + \gamma_A(x) \leq 1)$
Now $\theta_A(x) = (1 - (\mu_A(x) + \eta_A(x) + \gamma_A(x))$ could be called the degree of refusal membership of x in A.
Let PFS (X) denote the set of all the picture fuzzy sets on universe X.

**Definition 2.2 [3]:** A intuitionistic fuzzy set A on a universe X is an object of the form
$A = \{ (x, (\mu_A(x), \gamma_A(x))\} \mid x \in X) \}$
Where $\mu_A(x)$€ [0.1] is called the degree membership of x in A, $\gamma_A(x)$€ [0,1] is called the degree of non-membership of x in A, and where $\mu_A$ and $\gamma_A$ satisfy the following condition:
$(\forall x \in X)\ (\mu_A(x) + \gamma_A(x) \leq 1)$.

**Definition 2.3 [3]:** For every two PFSs A and B, the union, intersection and complement are defined as follows:

- $A \subseteq B$ iff ($\forall x \in X$, $\mu_A(x) \leq \mu_B(x)$ and $\eta_A(x) \leq \eta_B(x)$ and $\gamma_A(x) \geq \gamma_B(x)$)
- $A = B$ iff ($A \subseteq B$ and $B \subseteq A$)
- $A \cup B = \{(x, \max(\mu_A(x), \mu_B(x)), \min(\eta_A(x), \eta_B(x)), \min(\gamma_A(x), \gamma_B(x)) \mid x \in X\}$
- $A \cap B = \{(x, \min(\mu_A(x), \mu_B(x)), \min(\eta_A(x), \eta_B(x)), \max(\gamma_A(x), \gamma_B(x)) \mid x \in X)$
- $co(A) = \bar{A} = \{(\gamma_A(x), \eta_A(x), \mu_A(x)) \mid x \in X\}$

**Definition 2.5** [16]
The representation of a single-valued picture fuzzy set (SVPFS) N that exists within the set X is articulated as follows:

$$\gamma_A(x) = \begin{cases} \frac{p^\gamma - q_1^\gamma}{q_2^\gamma - q_1^\gamma} \; for\; q_1^\gamma \le p^\gamma \le q_2^\gamma \\ 1 \quad for\; q_2^\gamma \le p^\gamma \le q_3^\gamma \\ \frac{q_4{}^\gamma - p^\gamma}{q_4^\gamma - q_3^\gamma} \; for\; q_3^\gamma \le p^\gamma \le q_4^\gamma \\ 0 \quad for \quad otherwise \end{cases}$$

Where $q_1^\gamma \le q_2{}^\gamma \le q_3^\gamma \le q_4^\gamma$

$$\eta_A(x) = \begin{cases} \frac{p^\eta - q_1^\eta}{q_2^\eta - q_1^\eta} \; for\; q_1^\eta \le p^\eta \le q_2^\eta \\ 1 \quad for\; q_2^\eta \le p^\eta \le q_3^\eta \\ \frac{q_4{}^\eta - p^\eta}{q_4^\eta - q_3^\eta} \; for\; q_3^\eta \le p^\eta \le q_4^\eta \\ 1 \quad for \quad otherwise \end{cases}$$

Where $q_1^\eta \le q_2{}^\eta \le q_3^\eta \le q_4^\eta$

$$\mu_A(x) = \begin{cases} \frac{p^\mu - q_1^\mu}{q_2^\mu - q_1^\mu} \; for\; q_1^\mu \le p^\mu \le q_2^\mu \\ 1 \quad for\; q_2^\mu \le p^\mu \le q_3^\mu \\ \frac{q_4{}^\eta - p^\eta}{q_4^\eta - q_3^\eta} \; for\; q_3^\mu \le p^\mu \le q_4^\mu \\ 1 \quad for \quad otherwise \end{cases}$$

Where$q_1^\mu \le q_2{}^\mu \le q_3^\mu \le q_4^\mu$

**Definition 2.6** [16]

The definition of the (α, β, γ)-cut approach for SVTPN is articulated as follows:

$A_{\alpha,\beta,\gamma} = [\tilde{A}_1(\alpha), \tilde{A}_2(\alpha)]; [\tilde{A}_1{}'(\beta), \tilde{A}_2{}'(\beta)], [\tilde{A}_1(\gamma), \tilde{A}_2(\gamma)], 0 \le \alpha + \beta + \gamma \le 3$,where

$$[\tilde{A}_1(\alpha), \tilde{A}_2(\alpha)] = [(q_1{}^T + \alpha(q_2{}^T - q_1{}^T)), (q_3{}^T - \alpha(q_4{}^T - q_3{}^T))]$$

$$[\tilde{A}_1{}'(\beta), \tilde{A}_2{}'(\beta)] = [(q_1{}^I - \beta(q_2{}^I - q_1{}^I)), (q_3{}^I + \beta(q_4{}^I - q_3{}^I))]$$

$$[\tilde{A}_1{}''(\gamma), \tilde{A}_2{}''(\gamma)] = [(q_1{}^F - \gamma(q_2{}^F - q_1{}^F)), (q_3{}^F + \gamma(q_4{}^F - q_3{}^F))]$$

**Definition 2.7**[6]

Let us consider the two interval values denoted by ordered pairs of actual numbers with the lower and upper bounds be$F = [m_1, m_2], m_1 \le m_2 and H = [n_1, n_2], n_1 \le n_2$,with following properties:

$[m_1, m_2] + [n_1, n_2] = [m_1 + n_1, m_2 + n_2]$

$[m_1, m_2] - [n_1, n_2] = [m_1 - n_1, m_2 - n_2]$

$[m_1, m_2] \times [n_1, n_2] = [min(m_1n_1, m_1n_2, m_2n_1, m_2n_2), max(m_1n_1, m_1n_2, m_2n_1, m_2n_2)]$

$[m_1, m_2] \div [n_1, n_2] = [m_1, m_2] \times [1/n_1, 1/n_2]$, provided that 0 does not belong to$[n_1, n_2]$

$\alpha \times [m_1, m_2] = [\alpha m_1, \alpha m_2] for \alpha > 0 and [\alpha m_2, \alpha m_1]\; for\; \alpha < 0$

***The Picture Fuzzy Queuing Model(M/D/1)***

In this section, we are going to introduce a single server queue with deterministic service time in a picture fuzzy environment.

**Presumptions and Notations**

Corresponding as sumptions are used in the current model

i) M/D/1/∞/FCF Squeuing system with single server has an unbounded limit.

ii) Entry period that are distributed exponentially

iii)Deterministic utility period, i.e. fixed

iv) Entry and utility period are picture trapezoidal fuzzy numbersand symbolsarepresentedas:
μ=utilityperiod
λ= entryperiod
$N_s$=Expected no of consumer count in the system
$N_q$ =Expected no of consumers waiting in line
$W_s$ =System's average client hold time
$W_q$ =Expected time of an average consumer waits in queue
W = Customer Entry level
Z = Utility periodof Customer
A=entrylevel
S=utility level

**Suggested queuing miniature**

In this paper, we prefer (FCFS) first come first serve single server queuing model. In Kendall's notation, it is denoted as (M/D/1): (∞/FCFS).Here, D denotes picture fuzzy steady (constant) distribution with utility rate and M denotes picture fuzzy exponential distribution with entry rate. This stochastic process, which in cludes any entity currently engaged in administration, has a sequence with the values 0, 1, 2, 3...where the numbers denotes how many peoples currently utilizing the server. Its size is infinite, therefore there is no limitation to the no of clients it can accommodate.

Expected no of consumer count in the system:

$$N_{L_s} = \frac{\lambda}{\mu} + \frac{\lambda^2}{2\mu(\mu - \lambda)}$$

Expected no of consumers waiting in line:

$$N_{L_q} = \frac{\lambda^2}{2\mu(\mu - \lambda)}$$

Expected time of an average consumer waits in queue:

$$N_{W_q} = \frac{\lambda}{2\mu(\mu - \lambda)}$$

System's average client hold time:

$$N_{W_s} = \frac{1}{\mu} + \frac{\lambda}{2\mu(\mu - \lambda)}$$

**Solution procedure**

A simple method called DSW (Dong, Shah, and Wong) uses intervals at different α, β, γ-cut dimensions to describe execution proportions. The segregation extending on the picture fuzzy variable area prevents variance from the output membership function. Each ongoing involvement capacity can be addressed by an endless range of cuts with terms ranging from 0 to 1. Let $\mu_{A`}(\tilde{a})$&$\mu_{S`}(\tilde{s})$ be the membership performs of the utility period and the inter entry period. Intervals between entry period and utility period are shown as picture fuzzy sets as follows:

A` = $\{\tilde{a},\ \mu_{A`}(\tilde{a}),\ \eta_{A`}(\tilde{a}), \gamma_{A`}(\tilde{a}),\ \tilde{a} \in W\}$ and S` = $\{\tilde{s},\ \mu_{A`}(\tilde{s}),\ \eta_{A`}(\tilde{s}), \gamma_{A`}(\tilde{s}),\ \tilde{s} \in Z\}$
The α, β, γ-cuts of entry period, utility period are represented as:
A`(α)=$\{\mu_{A`}(\tilde{a}),\ \eta_{A`}(\tilde{a}), \gamma_{A`}(\tilde{a}) /\ \tilde{a} \in W\}$and
S` (α) = $\{\mu_{A`}(\tilde{s}),\ \eta_{A`}(\tilde{s}), \gamma_{A`}(\tilde{s})\ /\ \tilde{s} \in Z\}$
The DSW calculation contains the following steps:
Step 1: Define (α, β, γ)-cut value where $0 \le \alpha, \beta, \gamma \le 1$
Step 2: Next to compare with this α, β, γ find the membership performs intervals.
Step 3: The interval for the output membership perform is processed for the selected (α, β, γ) - cut measurements using common binary interval operation
Step 4: Repeat steps 1-3 for various estimates of α, β, γ.

**Formulation of M/D/1 queuing model**

Let's contemplate a queuing model featuring a solitary server M/D/1 configuration and deterministic service time. The picture fuzzy entry period $\bar{A}$, the approximate knowledge of picture fuzzy utility periods $\bar{S}$ is elucidated as follows:

$$\bar{A} = \{a, \mu_{\bar{A}}(a), \eta_{\bar{A}}(a), \gamma_{\bar{A}}(a) / \ a\epsilon W\}$$
$$\bar{S} = \{s, \mu_{\bar{A}}(s), \eta_{\bar{A}}(s), \gamma_{\bar{A}}(s) / \ s\epsilon Z\}$$

where $W$ and $Z$ represents crisp sets of the picture fuzzy entry period and picture fuzzy utility period and $\mu_{\bar{A}}(a), \mu_{\bar{S}}(s)$ are the corresponding membership performs. The $(\alpha, \beta, \gamma)$-cut of $\bar{A}$and $\bar{S}$ are

$$A(\alpha, \beta, \gamma) = \{a \in X / T_{\bar{A}}(a) \geq \alpha, I_{\bar{A}}(a) \leq \beta, F_{\bar{A}}(a) \leq \gamma\}$$
$$S(\alpha, \beta, \gamma) = \{s \in X / T_{\bar{S}}(s) \geq \alpha, I_{\bar{S}}(s) \leq \beta, F_{\bar{S}}(s) \leq \gamma\}$$

where $A(\alpha, \beta, \gamma)$ and $S(\alpha, \beta, \gamma)$ are the subsets of W and Z respectively. Here we can represent picture fuzzy inter entry time and picture fuzzy overhauled time by the different levels of intervals using $(\alpha, \beta, \gamma)$-cuts. As a result, a picture fuzzy queue can be transformed into a collection of crisp queues, each characterized by distinct $(\alpha, \beta, \gamma)$-cuts.

$$\{A(\alpha, \beta, \gamma): 0 < \alpha \leq 1, 0 \leq \beta < 1, 0 \leq \gamma < 1\}$$
$$\{S(\alpha, \beta, \gamma): 0 < \alpha \leq 1, 0 \leq \beta < 1, 0 \leq \gamma < 1\}.$$

In this paper, we proposed a queuing model with both inter entry time $\bar{A}$and overhauled time $\bar{S}$are defined as SVTPN and the intervals of $\bar{A}$and $\bar{S}$are denoted by $[l_{\bar{A}(\alpha,\beta,\gamma)}, u_{\bar{A}(\alpha,\beta,\gamma)}]$ and $[l_{\bar{S}(\alpha,\beta,\gamma)}, u_{\bar{S}(\alpha,\beta,\gamma)}]$. Following the application of Zadeh's extension principle, we can define the positive(truth) membership function, neutral membership function and negative(false) membership function of the performance measure P as follows.

$$T_{P(\bar{A},\bar{S})}(w) = sup\left\{min_{a\in W, a'\in Z}\left(\mu_{\bar{A}(a)}, \mu_{S(a')}\right) : w = p(a, a')\right\}$$
$$I_{P(\bar{A},S)}(w) = inf\left\{min_{a\in W, a'\in Z}\left(\mu_{\bar{A}(a)}, \mu_{\bar{S}(a')}\right) : w = p(a, a')\right\}$$
$$F_{P(\bar{A},\bar{S})}(w) = inf\left\{min_{a\in W, a'\in Z}\left(\mu_{\bar{A}(a)}, \mu_{\bar{S}(a')}\right) : w = p(a, a')\right\}$$

We determine the lower and upper bounds of the $(\alpha, \beta, \gamma)$ cuts of $(\bar{A}, \bar{S})$ as follows;

$$l_{P(\alpha,\beta,\gamma)} = min\, p\,(a, a') \ni l_{\bar{A}(\alpha,\beta,\gamma)} \leq a \leq u_{\bar{A}(\alpha,\beta,\gamma)}, l_{\bar{S}(\alpha,\beta,\gamma)} \leq a' \leq u_{\bar{S}(\alpha,\beta,\gamma)} \quad (1)$$
$$u_{P(\alpha,\beta,\gamma)} = max\, p\,(a, a') \ni l_{\bar{A}(\alpha,\beta,\gamma)} \leq a \leq u_{\bar{A}(\alpha,\beta,\gamma)}, l_{\bar{S}(\alpha,\beta,\gamma)} \leq a' \leq u_{\bar{S}(\alpha,\beta,\gamma)} \quad (2)$$

Provided $a \in \overline{A}(\alpha, \beta, \gamma)$ and $a' \in \overline{S}(\alpha, \beta, \gamma)$. If both $l_{P(\alpha,\beta,\gamma)}$ and $u_{P(\alpha,\beta,\gamma)}$ are invertible with respect to $(\alpha, \beta, \gamma)$ then the left shape function$L_T(w) = \left(l_{P(\alpha,\beta,\gamma)}\right)^{-1}$and right shape functions $R_T(w) = \left(u_{P(\alpha,\beta,\gamma)}\right)^{-1}$ may be constructed and the truth membership function $T_{P(\bar{A},\bar{S})}(w)$ is obtained by

$$T_{P(\bar{A},\bar{S})}(w) = \begin{cases} L_T(w); {w_1}^T \leq w \leq {w_2}^T \\ R_T(w); {w_3}^T \leq w \leq {w_4}^T \\ 0; otherwise \end{cases}$$

where ${w_1}^T \leq w \leq {w_4}^T$ and $L_T({w_1}^{T)} = R_T({w_4}^T) = 0$ for the SVTPN.

Similarly, the neutral membership function $I_{P(\bar{A},\bar{S})}(w)$ and the falsity membership function$F_{P(\bar{A},\bar{S})}(w)$, are derived in the same way,

$$I_{P(\bar{A},\bar{S})}(w) = \begin{cases} L_T(w); {w_1}^T \leq w \leq {w_2}^T \\ R_T(w); {w_3}^T \leq w \leq {w_4}^T \\ 0; otherwise \end{cases}$$

where $w_1{}^I \le w \le w_4{}^I$ and $L_I(w_1{}^I) = R_I(w_4{}^I) = 0$ for the SVTPN.

$$F_{P(\bar{A},\bar{S})}(w) = \begin{cases} L_F(w); w_1{}^F \le w \le w_2{}^F \\ R_F(w); w_3{}^F \le w \le w_4{}^F \\ 0; otherwise \end{cases}$$

where $w_1{}^F \le w \le w_4{}^F$ and $L_F(w_1{}^F) = R_F(w_4{}^F) = 0$ for the SVTPN.

By applying $(\alpha, \beta, \gamma)$ - cut approach, the proposed M/D/1 queue with deterministic service time can be streamlined into a conventional M/D/1 queue

**Numerical demonstration**

Let's examine an M/D/1:∞/FCFS model where both the entry rate and utility rate are represented by single valued trapezoidal picture fuzzy number and from equations (1) & (2) the membership functions of$\bar{N}_{L_q}$, $\bar{N}_{L_S}$and $\bar{N}_{w_q}$, $\bar{N}_{w_s}$ are listed below.

Let the arrival and service rates are represented by

$\bar{A}$ = {($x_1$, 0.2,0.0,0.2,0.6),($x_2$, 0.7, 0.0, 0.1, 0.2), ($x_3$, 0.5, 0.0, 0.4, 0.1)}

$\bar{S}$= {($x_1$, 0.3,0.0,0.0,0.7),($x_2$, 0.7, 0.0, 0.3, 0.0), ($x_3$, 0.5, 0.0, 0.5, 0.0)}

The (α, β, γ)-cut of $\bar{A}$ and $\bar{S}$ are

$\bar{A}$ = [0.2 + α, 0.6 – α ], [0- β, 0.1 + β], [ 0 – γ, 0.4 + γ]

$\bar{S}$ = [0.3 + α, 0.7 – α ], [0- β, 0.3 + β], [ 0 – γ, 0.5 + γ]

Now,

$$l_{L_q}(\alpha) = min\left\{\frac{e_1{}^2}{2e_2(e_2-e_1)}\right\} \quad (3)$$

and

$$u_{L_q}(\alpha) = max\left\{\frac{e_1{}^2}{2e_2(e_2-e_1)}\right\} \quad (4)$$

Such that $0.2 + \alpha < e_1 < 0.6 - \alpha$

$0.3 + \alpha < e_2 < 0.7 - \alpha$

where$0 < \alpha \le 1$, $l_{L_q}(\alpha)$is discovered while $e_1$ approaching their lower bound (l.b) and $e_2$ approaching its upper bound (u.b) and as well as $u_{L_q}(\alpha)$ is discovered while $e_1$ approaching their u.b and $e_2$ approaching its l.b. As a result the best solution for (3) and (4) is

$$l_{L_q}(\propto) = \frac{\propto^2+0.4\propto+0.04}{4\propto^2-3.8\propto+0.7}; \quad u_{L_q}(\propto) = \frac{\propto^2-1.2\propto+0.36}{4\propto^2+0.6\propto-0.18};$$

The truth membership function is

$$T_{\bar{N}_{L_q}}(w) = \begin{cases} L_T(w); & -4.57 \le w \le 4.54 \\ R_T(w); & -3.125 \le w \le 1.6 \\ 0; & otherwise \end{cases}$$

$$l_{L_q}(\beta) = min\left\{\frac{e_1{}^2}{2e_2(e_2-e_1)}\right\} \quad (5)$$

and

$$u_{L_q}(\beta) = max\left\{\frac{e_1{}^2}{2e_2(e_2-e_1)}\right\} \quad (6)$$

Such that $0 - \beta < e_1 < 0.1 + \beta$

$0 - \beta < e_2 < 0.3 + \beta$

where$0 < \alpha \le 1$, $l_{L_q}(\beta)$is discovered while $e_1$ approaching their lower bound (l.b) and$e_2$ approaching its upper bound (u.b) and as well as $u_{L_q}(\beta)$ is discovered while $e_1$ approaching their u.b and $e_2$ approaching its l.b. As a result the best solution for (5) and (6) is

$$l_{L_q}(\beta) = \frac{\beta^2}{4\beta^2+1.8\beta+0.18}; \quad u_{L_q}(\beta) = \frac{\beta^2+0.2\beta+0.01}{4\beta^2+0.2\beta};$$

$$I_{\bar{N}_{L_q}}(w) = \begin{cases} L_I(w); & 0 \le w \le 0.1672 \\ R_I(w); & 0 \le w \le 0.67 \\ 0; & otherwise \end{cases}$$

$$l_{L_q}(\gamma) = min\left\{\frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (7)$$

and

$$u_{L_q}(\gamma) = max\left\{\frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (8)$$

Such that $0-\gamma < e_1 < 0.4+\gamma$

$0-\gamma < e_2 < 0.5+\gamma$

where $0 < \alpha \le 1$, $l_{L_q}(\gamma)$is discovered while $e_1$ approaching their lower bound (l.b) and$e_2$ approaching its upper bound (u.b) and as well as $u_{L_q}(\gamma)$ is discovered while $e_1$ approaching their u.b and $e_2$ approaching its l.b. As a result the best solution for (7) and (8) is

$$l_{L_q}(\gamma) = \frac{\gamma^2}{4\gamma^2+3\gamma+0.5};\ u_{L_q}(\gamma) = \frac{\gamma^2+0.2\gamma+1.6}{4\gamma^2+0.8\gamma};$$

The falsity membership function is

$$F_{\bar{N}_{L_q}}(w) = \begin{cases} L_F(w); & 0 \le w \le 0.13 \\ R_F(w); & 0 \le w \le 2.91 \\ 0; & otherwise \end{cases}$$

We have calculated the expected number of consumers waiting in line for various values of α, β, and γ, and the results are presented in Table 1.

Additionally, we have provided graphical representations of truth, indeterminacy and falsity of $\bar{N}_{L_q}$ is shown in figures. 1, 2 and 3.

Similarly the performance measure of $\bar{\boldsymbol{N}}_{\boldsymbol{L_S}}$for α-cut is given below,

$$l_{L_S}(\alpha) = min\left\{\frac{e_1}{e_2} + \frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (9)$$

and

$$u_{L_S}(\alpha) = max\left\{\frac{e_1}{e_2} + \frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (10)$$

The objective functions for $\bar{N}_{L_S}$ isgiven through the equations (9) and (10)

$$l_{L_s}(\propto) = \frac{3\propto^3-2.7\propto^2+0.18\propto+0.168}{-4\propto^3+6.6\propto^2-3.36\propto+0.49};\quad u_{L_s}(\propto) = \frac{-3\propto^3+0.9\propto^2+0.54\propto-0.108}{4\propto^3+1.8\propto^2-0.054};$$

$$T_{\bar{N}_{L_s}}(w) = \begin{cases} L_T(w); & -5.445 \le w \le 2.4 \\ R_T(w); & -0.25 \le w \le 2 \\ 0; & otherwise \end{cases}$$

Similarly the performance measure of $\bar{N}_{L_S}$for β-cut is given below,

$$l_{L_S}(\beta) = min\left\{\frac{e_1}{e_2} + \frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (11)$$

and

$$u_{L_S}(\beta) = max\left\{\frac{e_1}{e_2} + \frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (12)$$

The objective functions for $\bar{N}_{L_s}$ is given through the equations (11) and (12)

$$l_{L_s}(\beta) = \frac{-3\beta^3-1.5\beta^2-0.18\beta}{4\beta^3+3\beta^2+0.72\beta+0.054};\quad u_{L_s}(\beta) = \frac{3\beta^2+0.4\beta+0.01}{-4\beta^2-0.2\beta};$$

$$I_{\bar{N}_{L_s}}(w) = \begin{cases} L_I(w); & -0.602 \le w \le 0 \\ R_I(w); & -0.81 \le w \le 0 \\ 0; & otherwise \end{cases}$$

Similarly the performance measure of $\bar{N}_{L_s}$for γ-cut is given below,

$$l_{L_S}(\gamma) = max\left\{\frac{e_1}{e_2} + \frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (13)$$

and

$$u_{L_S}(\gamma) = max\left\{\frac{e_1}{e_2} + \frac{{e_1}^2}{2e_2(e_2-e_1)}\right\} \quad (14)$$

The objective functions for $\bar{N}_{L_s}$ is given through the equations (13) and (14)

$l_{L_s}(\gamma) = \frac{-3\gamma^3-2.5\gamma^2-0.5\gamma}{4\gamma^3+5\gamma^2+2\gamma+0.25}$; $u_{L_s}(\gamma) = \frac{-3\gamma^2-2.2\gamma+1.28}{4\gamma^2+0.8\gamma}$ ;

$$F_{\bar{N}_{L_s}}(w) = \begin{cases} L_F(w); & -0.53 \le w \le 0 \\ R_F(w); & -0.8 \le w \le 8.5 \\ 0; & otherwise \end{cases}$$

We have computed the expected number of consumers in the system for varying values of α, β, γ∈ [0,1]and these results are presented in Table 2.

Additionally, Figures 4, 5, and 6 present a graphical representation of the truth, indeterminacy, and falsity of $\bar{N}_{L_S}$ is being discussed.

Similarly the performance measure of $\bar{N}_{W_q}$for α-cut is given below,

$$l_{Wq}(\alpha) = min\left\{\frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (15)$$

and

$$u_{Wq}(\alpha) = max\left\{\frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (16)$$

The objective functions for$\bar{N}_{W_q}$ is given through the equations (15) and (16)

$l_{w_q}(\propto) = \frac{0.2+\propto}{4\propto^2-3.8\propto+0.7}$; $u_{w_q}(\propto) = \frac{0.6-\propto}{4\propto^2+0.6\propto-0.18}$;

$$T_{\bar{N}_{L_{w_q}}}(w) = \begin{cases} L_T(w); & -6.25 \le w \le 4.5454 \\ R_T(w); & -3.333 \le w \le 0.8333 \\ 0; & otherwise \end{cases}$$

similarly the performance measure of $\bar{N}_{W_q}$for β-cut is given below,

$$l_{Wq}(\beta) = min\left\{\frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (17)$$

and

$$u_{Wq}(\beta) = max\left\{\frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (18)$$

The objective functions for $\bar{N}_{W_q}$is given through the equations (17) and (18)

$l_{w_q}(\beta) = \frac{-\beta}{4\beta^2+1.8\beta+0.18}$; $u_{w_q}(\beta) = \frac{0.1+\beta}{4\beta^2+0.2\beta}$;

$$I_{\bar{N}_{L_{w_q}}}(w) = \begin{cases} L_I(w); & -0.2857 \le w \le 0 \\ R_I(w); & 0.1467 \le w \le 0.2381 \\ 0; & otherwise \end{cases}$$

similarly the performance measure of $\bar{N}_{W_q}$for γ-cut is given below,

$$l_{Wq}(\gamma) = min\left\{\frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (19)$$

and

$$u_{Wq}(\gamma) = max\left\{\frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (20)$$

The objective functions for $\bar{N}_{W_q}$ is given through the equations (19) and (20)

$l_{w_q}(\gamma) = \frac{-\gamma}{4\gamma^2+3\gamma+0.5}$; $u_{w_q}(\gamma) = \frac{0.4+\gamma}{4\gamma^2+0.8\gamma}$;

$$F_{\bar{N}_{L_{w_q}}}(w) = \begin{cases} L_F(w); & -0.2 \le w \le 0 \\ R_F(w); & 0 \le w \le 4.17 \\ 0; & otherwise \end{cases}$$

We have performed calculations for the expected time an average customer waits in the queue, considering different values of α, β, and γ, and the results are documented in Table 3. Furthermore, Figures 7, 8, and 9 depict a graphical examination of the truth, indeterminacy, and falsity aspects of $\bar{N}_{W_q}$ is discussed.

Similarly the performance measure of $\bar{N}_{W_s}$for α-cut is given below,

$$l_{Ws}(\alpha) = min\left\{\frac{1}{e_2} + \frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (21)$$

and

$$u_{W_S}(\alpha) = max\left\{\frac{1}{e_2} + \frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (22)$$

The objective functions for $\bar{N}_{W_S}$ is given through the equations (21) and (22)

$$l_{w_s}(\propto) = \frac{3\propto^2-3.3\propto+0.84}{-4\propto^3+6.6\propto^2-3.36\propto+0.49}; \quad u_{w_s}(\propto) = \frac{3\propto^2+0.9\propto}{4\propto^3+1.8\propto^2-0.054};$$

$$T_{\bar{N}_{w_s}}(w) = \begin{cases} L_T(w); & -5.4545 \le w \le 6 \\ R_T(w); & -3.75 \le w \le 2.75 \\ 0; & otherwise \end{cases}$$

similarly the performance measure of $\bar{N}_{W_S}$ for β-cut is given below,

$$l_{W_S}(\beta) = min\left\{\frac{1}{e_2} + \frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (23)$$

and

$$u_{W_S}(\beta) = max\left\{\frac{1}{e_2} + \frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (24)$$

The objective functions for $\bar{N}_{W_S}$ is given through the equations (23) and (24)

$$l_{w_s}(\beta) = \frac{3\beta^2+1.5\beta+0.18}{4\beta^3+3\beta^2+0.72\beta+0.054}; \quad u_{w_s}(\beta) = \frac{3\beta+0.1}{-4\beta^2-0.2\beta};$$

$$I_{\bar{N}_{w_s}}(w) = \begin{cases} L_I(w); & 0.6020 \le w \le 3.333 \\ R_I(w); & -6.667 \le w \le 0 \\ 0; & otherwise \end{cases}$$

similarly the performance measure of $\bar{N}_{W_S}$ for γ-cut is given below,

$$l_{W_S}(\gamma) = min\left\{\frac{1}{e_2} + \frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (25)$$

and

$$u_{W_S}(\gamma) = max\left\{\frac{1}{e_2} + \frac{e_1}{2e_2(e_2-e_1)}\right\} \quad (26)$$

The objective functions for $\bar{N}_{W_S}$ is given through the equations (25) and (26)

$$l_{w_s}(\gamma) = \frac{3\gamma^2+2.5\gamma+0.5}{4\gamma^3+5\gamma^2+2\gamma+0.25}; \quad u_{w_s}(\gamma) = \frac{3\gamma+0.4}{-4\gamma^2-0.8\gamma};$$

$$F_{\bar{N}_{w_s}}(w) = \begin{cases} L_F(w); & 0.533 \le w \le 1.5476 \\ R_F(w); & -5.833 \le w \le -0.708 \\ 0; & otherwise \end{cases}$$

The system's average client hold time has been computed for various values of α, β, and γ, and the outcomes are presented in Table 4.Also a graphical analysis of truth, indeterminacy and falsity of $\bar{N}_{W_S}$ is shown in figures. 10, 11 and 12.

## CONCLUSION

In this paper, we have successfully introduced and applied Picture Fuzzy Sets (PFS) to the M/D/1 queuing model, providing a novel approach to capturing and analyzing the uncertainties in queuing systems. Our hypothesis suggested that integrating PFS into the queuing model offers a more nuanced understanding of the relationships between the arrival process, service process, and customer behavior in the queue. This hypothesis was validated through the derivation of performance measures within a picture fuzzy environment. Both the entry level (arrival rate) and the utility period (service time) were expressed using Single Valued Trapezoidal Picture Fuzzy Numbers (SVTPN). This representation allowed us to effectively manage and analyze the inherent uncertainties in these parameters. We demonstrated our model with a numerical example.

Our analysis revealed that using PFS in queuing models not only captures the traditional uncertainties but also provides a richer framework to represent and evaluate the degrees of membership, non-membership, and hesitation that are typical in such systems. This leads to a more comprehensive understanding of the system dynamics and performance. Looking ahead, our study opens the door to further research and development in the field of fuzzy set theory applied to queuing systems. Future work could explore the extension of this approach to more advanced fuzzy set models, such as Type-II fuzzy sets and Pythagorean fuzzy sets, which can capture even higher levels of uncertainty and complexity. These advanced models could provide deeper insights and more precise performance

metrics in queuing theory and other operational research areas. In summary, the integration of Picture Fuzzy Sets into the M/D/1 queuing model represents a significant advancement in the modeling and analysis of queuing systems. This work lays a foundation for future explorations into more sophisticated fuzzy set applications, promising to broaden the scope and impact of fuzzy logic in both theoretical and practical domains.

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**Table 1. $\alpha$, $\beta$, $\gamma$ cuts for expected no of consumers in the queue**

| $\alpha$ | $l_{Lq}$ ($\alpha$) | $u_{Lq}$ ($\alpha$) | $\beta$ | $l_{Lq}$ ($\beta$) | $u_{Lq}$ ($\beta$) | $\Upsilon$ | $l_{Lq}$ ($\Upsilon$) | $u_{Lq}$ ($\Upsilon$) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.057142857 | -2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | 0.25 | -3.125 | 0.1 | 0.025 | 0.666666667 | 0.1 | 0.01 | 13.58333333 |
| 0.2 | 1.6 | 1.6 | 0.2 | 0.057142857 | 0.45 | 0.2 | 0.03 | 5.25 |
| 0.3 | -3.125 | 0.25 | 0.3 | 0.083333333 | 0.380952381 | 0.3 | 0.05 | 2.916666667 |
| 0.4 | -2 | 0.057142857 | 0.4 | 0.103896104 | 0.347222222 | 0.4 | 0.07 | 1.916666667 |
| 0.5 | -2.45 | 0.008928571 | 0.5 | 0.120192308 | 0.327272727 | 0.5 | 0.08 | 1.392857143 |
| 0.6 | -4.57142857 | 0 | 0.6 | 0.133333333 | 0.314102564 | 0.6 | 0.1 | 1.083333333 |
| 0.7 | 0 | 0.004545455 | 0.7 | 0.144117647 | 0.304761905 | 0.7 | 0.11 | 0.884920635 |
| 0.8 | 4.545454545 | 0.013986014 | 0.8 | 0.153110048 | 0.297794118 | 0.8 | 0.12 | 0.75 |
| 0.9 | 2.326923077 | 0.025 | 0.9 | 0.160714286 | 0.292397661 | 0.9 | 0.13 | 0.654040404 |
| 1 | 1.6 | 0.036199095 | 1 | 0.16722408 | 0.288095238 | 1 | 0.13 | 0.583333333 |

**Table .2. $\alpha$, $\beta$, $\gamma$ cuts for expected no of consumers in the system**

| $\alpha$ | $l_{Lq}$ ($\alpha$) | $u_{Lq}$ ($\alpha$) | $\beta$ | $l_{Lq}$ ($\beta$) | $u_{Lq}$ ($\beta$) | $\Upsilon$ | $l_{Lq}$ ($\Upsilon$) | $u_{Lq}$ ($\Upsilon$) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.34285714 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | 0.75 | 1.5 | 0.1 | -0.225 | -0.7333333 | 0.1 | -0.1547619 | 8.58333333 |
| 0.2 | 2.4 | 0.24 | 0.2 | -0.34285714 | -0.73 | 0.2 | -0.2539682 | 2.25 |
| 0.3 | -1.875 | 0.25 | 0.3 | -0.41666667 | -0.7523809 | 0.3 | -0.3238636 | 0.58333333 |
| 0.4 | 5.1399E-16 | 0.12244898 | 0.4 | -0.46753246 | -0.7694444 | 0.4 | -0.3760683 | -0.08333333 |
| 0.5 | 1.05 | 0.01339285 | 0.5 | -0.50480769 | -0.7818181 | 0.5 | -0.4166666 | -0.40714285 |
| 0.6 | 3.42857142 | -0.07407407 | 0.6 | -0.53333333 | -0.7910256 | 0.6 | -0.4491978 | -0.58333333 |
| 0.7 | 0 | -0.14454545 | 0.7 | -0.55588235 | -0.7980952 | 0.7 | -0.4758771 | -0.68650793 |
| 0.8 | -5.45454545 | -0.20216147 | 0.8 | -0.57416267 | -0.8036764 | 0.8 | -0.4981685 | -0.75 |
| 0.9 | -3.17307692 | -0.25 | 0.9 | -0.58928571 | -0.8081871 | 0.9 | -0.5170807 | -0.79040404 |
| 1 | -2.4 | -0.29028889 | 1 | -0.60200668 | -0.8119047 | 1 | -0.5333333 | -0.81666666 |

**Table.3. $\alpha$, $\beta$, $\gamma$ cuts for expected no of consumers waiting in queue**

| $\alpha$ | $l_{Lq}$ ($\alpha$) | $u_{Lq}$ ($\alpha$) | $\beta$ | $l_{Lq}$ ($\beta$) | $u_{Lq}$ ($\beta$) | $\Upsilon$ | $l_{Lq}$ ($\Upsilon$) | $u_{Lq}$ ($\Upsilon$) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.28571428 | -3.33333333 | 0 | 0 | 0.2 | 0 | 0 | 0 |
| 0.1 | 0.833333333 | -6.25 | 0.1 | -0.25 | 0.238095238 | 0.1 | -0.1 | 4.17 |
| 0.2 | 4 | 4 | 0.2 | -0.2857142 | 0.238095238 | 0.2 | -0.2 | 1.88 |
| 0.3 | -6.25 | 0.833333333 | 0.3 | -0.2777777 | 0.227272727 | 0.3 | -0.2 | 1.17 |
| 0.4 | -3.33333333 | 0.285714286 | 0.4 | -0.2597402 | 0.213675214 | 0.4 | -0.2 | 0.83 |
| 0.5 | -3.5 | 0.089285714 | 0.5 | -0.2403846 | 0.2 | 0.5 | -0.2 | 0.64 |
| 0.6 | -5.71428571 | 0 | 0.6 | -0.2222222 | 0.187165775 | 0.6 | -0.2 | 0.52 |
| 0.7 | 0 | -0.04545454 | 0.7 | -0.2058823 | 0.175438596 | 0.7 | -0.2 | 0.44 |
| 0.8 | 4.545454545 | -0.06993007 | 0.8 | -0.1913875 | 0.164835165 | 0.8 | -0.1 | 0.38 |
| 0.9 | 2.115384615 | -0.08333333 | 0.9 | -0.1785714 | 0.155279503 | 0.9 | -0.1 | 0.33 |
| 1 | 1.333333333 | -0.09049773 | 1 | -0.1672240 | 0.146666667 | 1 | -0.1 | 0.29 |

**Table.4 $\alpha$, β, γ cuts system's average client hold time**

| $\alpha$ | $l_{Lq}$ ($\alpha$) | $u_{Lq}$ ($\alpha$) | β | $l_{Lq}$ (β) | $u_{Lq}$ (β) | ϒ | $l_{Lq}$ (ϒ) | $w_{Lq}$ (ϒ) |
|---|---|---|---|---|---|---|---|---|
| 0 | 1.714285714 | 0 | 0 | 3.333333333 | 0 | 0 | 2 | 0 |
| 0.1 | 2.5 | -3.75 | 0.1 | 2.25 | -6.6666666 | 0.1 | 1.547619048 | -5.833333 |
| 0.2 | 6 | 6 | 0.2 | 1.714285714 | -3.5 | 0.2 | 1.26984127 | -3.125 |
| 0.3 | -3.75 | 2.5 | 0.3 | 1.388888889 | -2.3809523 | 0.3 | 1.079545455 | -2.166666 |
| 0.4 | 0 | 1.714285714 | 0.4 | 1.168831169 | -1.8055555 | 0.4 | 0.94017094 | -1.666666 |
| 0.5 | 1.5 | 1.339285714 | 0.5 | 1.009615385 | -1.4545454 | 0.5 | 0.833333333 | -1.357142 |
| 0.6 | 4.285714286 | 1.111111111 | 0.6 | 0.88888888 | -1.2179487 | 0.6 | 0.748663102 | -1.145833 |
| 0.7 | 0 | 0.954545455 | 0.7 | 0.794117647 | -1.0476190 | 0.7 | 0.679824561 | -0.992063 |
| 0.8 | -5.45454545 | 0.839160839 | 0.8 | 0.717703349 | -0.9191176 | 0.8 | 0.622710623 | -0.875 |
| 0.9 | -2.88461538 | 0.75 | 0.9 | 0.654761905 | -0.8187134 | 0.9 | 0.574534161 | -0.782828 |
| 1 | -2 | 0.678733032 | 1 | 0.602006689 | -0.7380952 | 1 | 0.533333333 | -0.708333 |

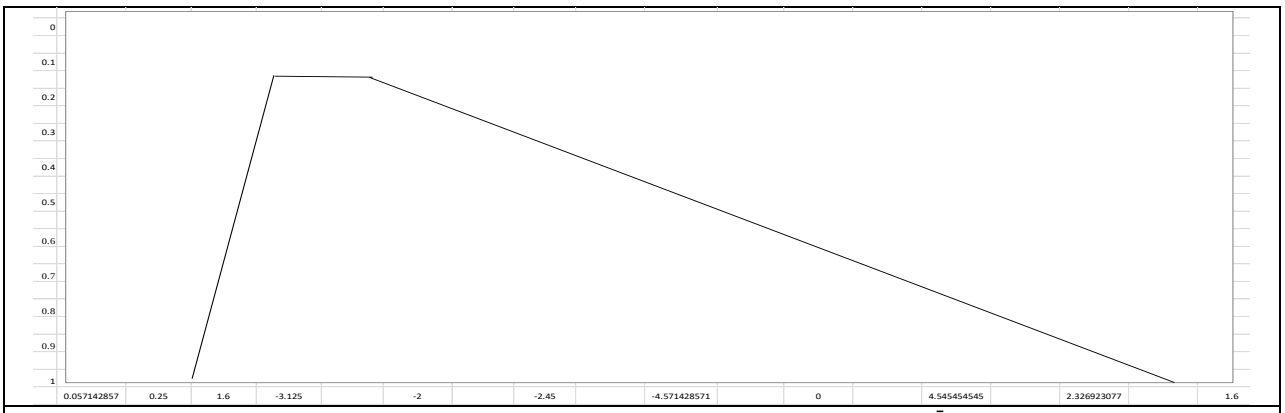


**Figure .1 Truth me mbership value for $\bar{N}_{L_q}$**

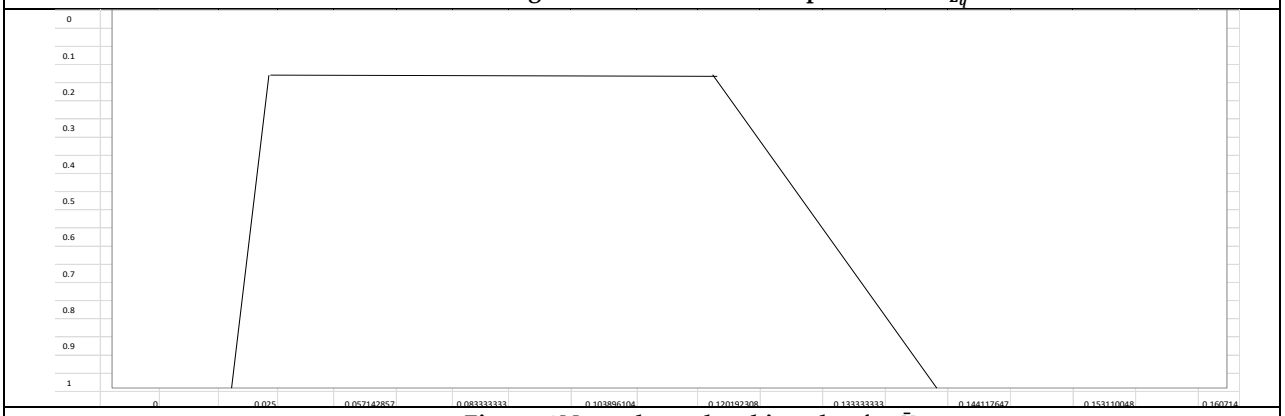


**Figure .2Neutral membership value for $\bar{N}_{L_q}$**

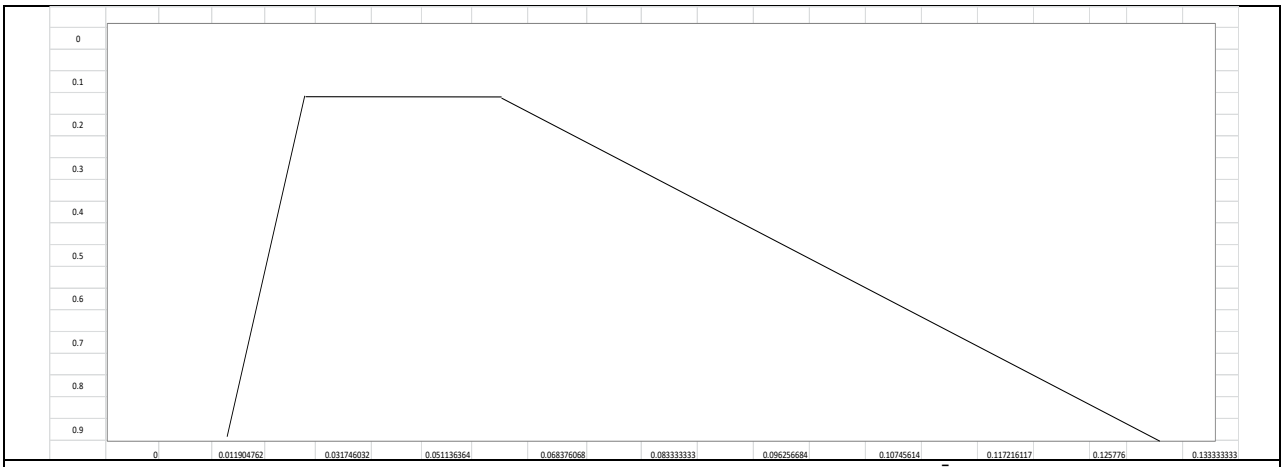


**Figure .3 Falsity membership value for $\bar{N}_{L_q}$**

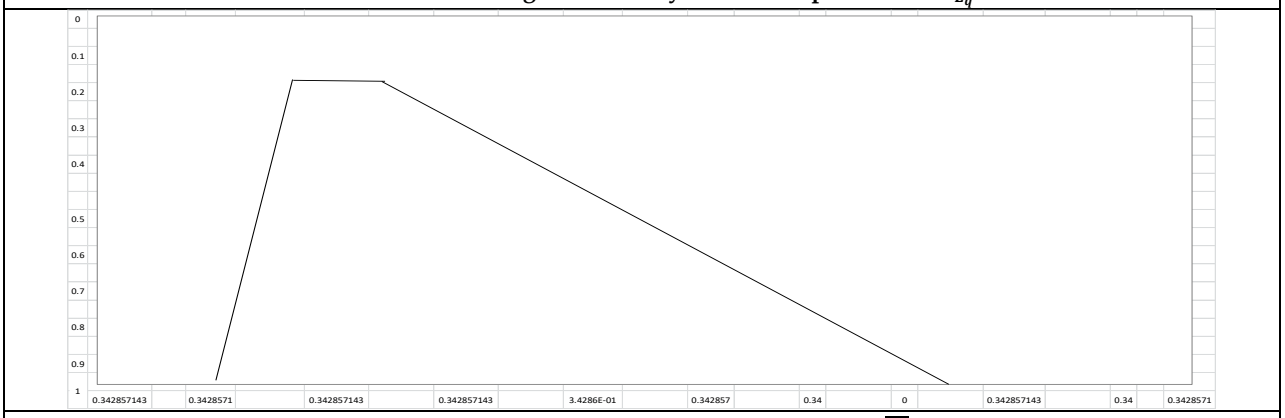


**Figure .4 Truth membership value for $\overline{N}_{L_S}$**

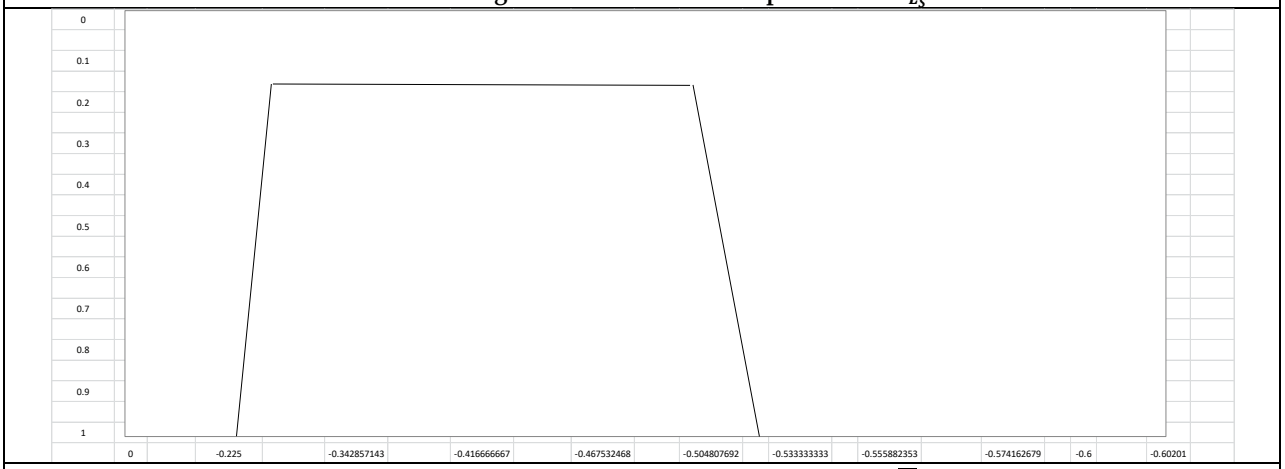


**Figure .5 Neutral membership value for$\overline{N}_{L_S}$**

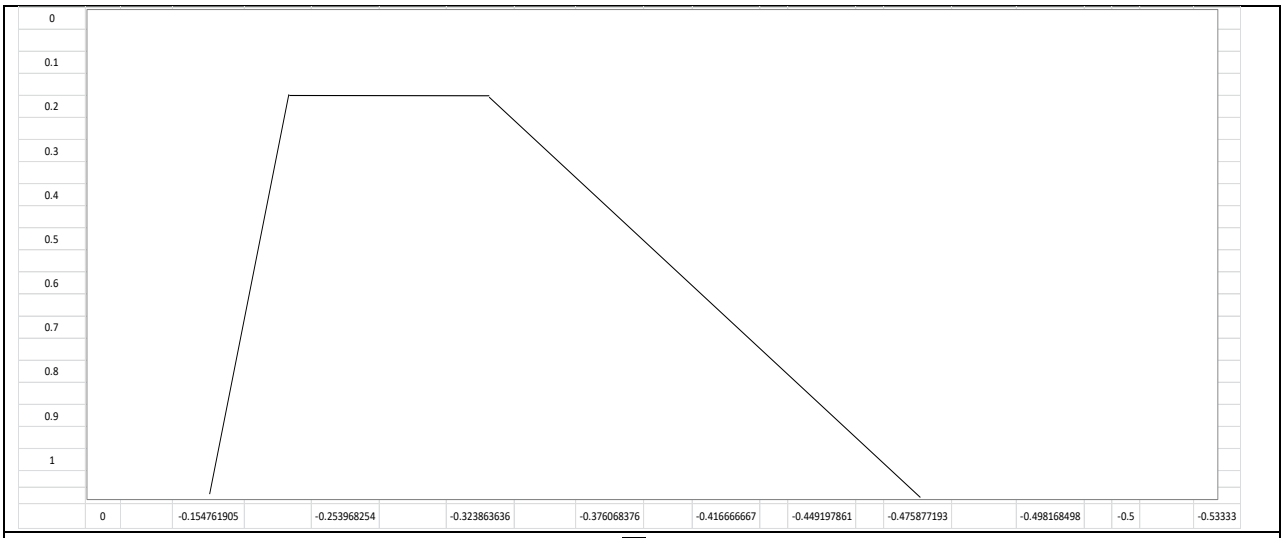


**Figure .6** Falsity membership value for $\overline{N}_{L_S}$

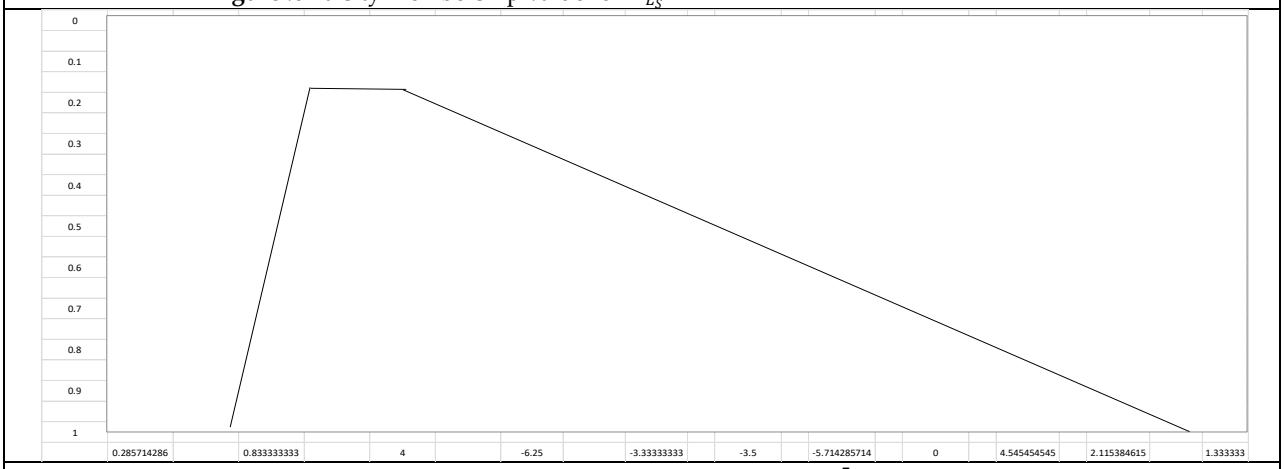


**Figure .7 Truth membership value for $\bar{N}_{W_q}$**

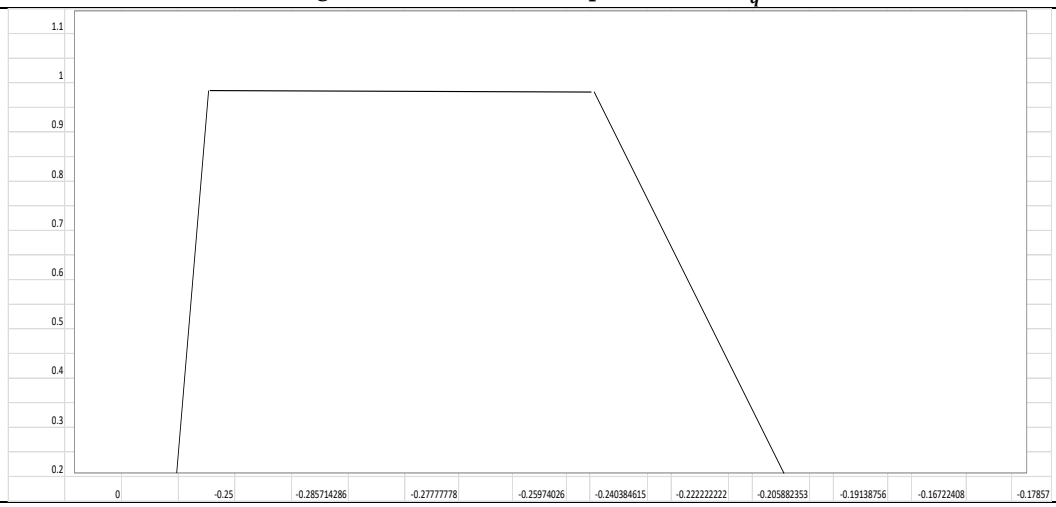


**Figure .8 Neutral membership value for $\bar{N}_{W_q}$**

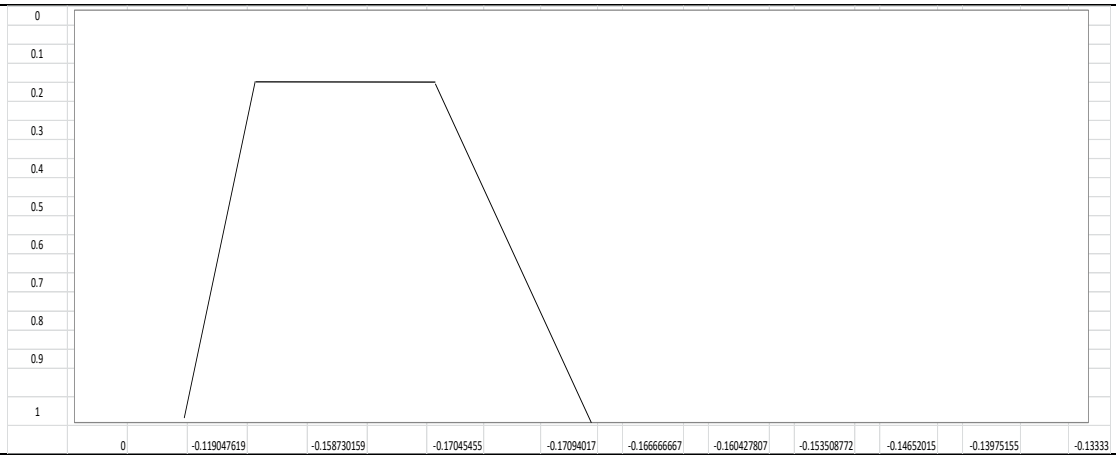


**Figure .9 Falsity membership value for $\bar{N}_{W_q}$**

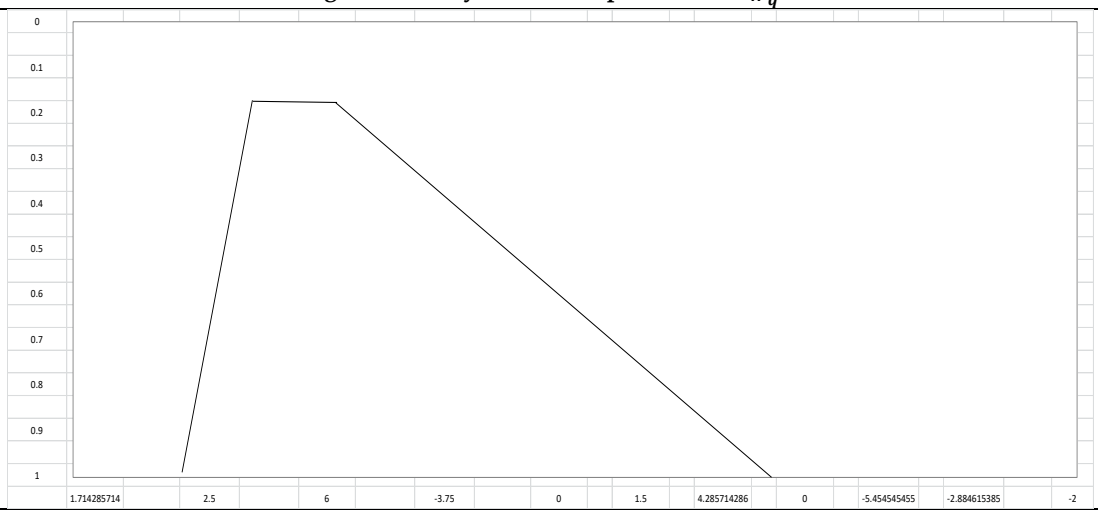


**Figure .10 Truth membership value for $\bar{N}_{W_s}$**

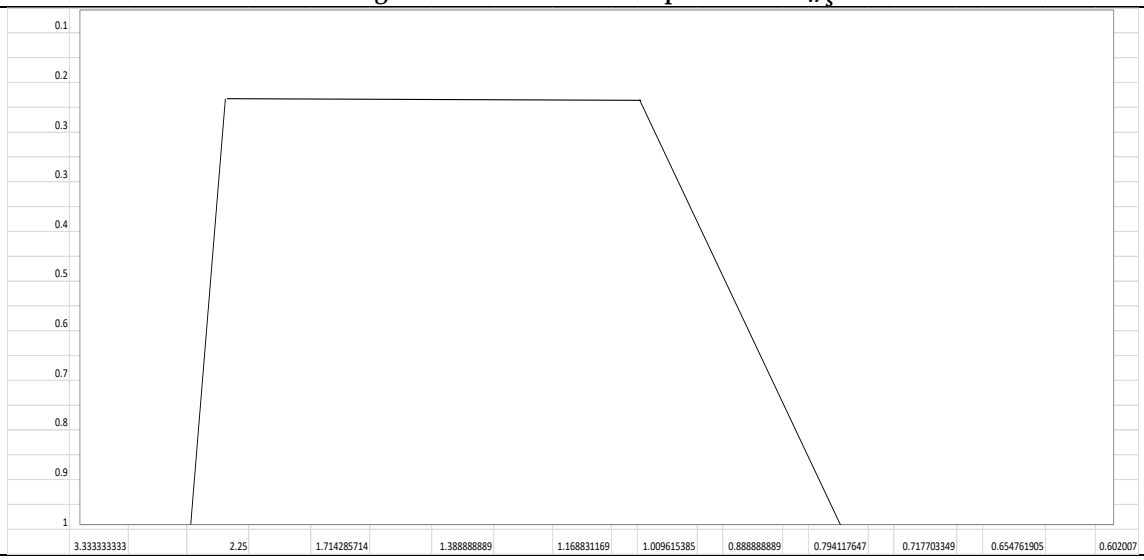


**Figure .11 Neutral membership value for $\bar{N}_{W_s}$**

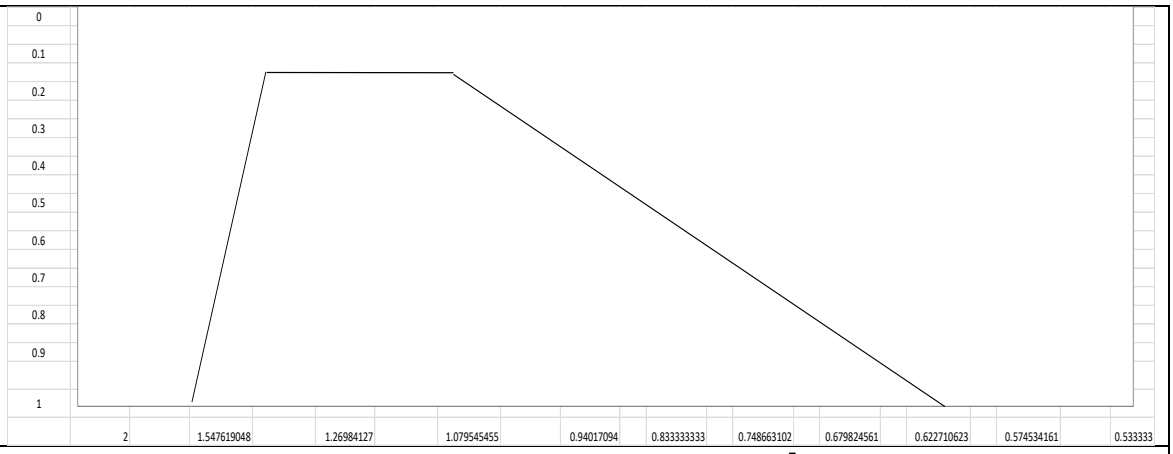


**Figure.12 Falsity membership value for $\bar{N}_{W_S}$**

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**RESEARCH ARTICLE**

# Preparation and Evaluation of Polyherbal Tea Bags with Anti-Oxidant Activity for Management of Oxidative Stress

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## ABSTRACT

Tea in general and herbal tea in particular, are gaining increasing consumer attention due to a growing awareness of health benefits derived from their consumption. Even though several underutilized plants exist with potential for processing into herb tea, research in product development of herb teas is limited. This research was conducted to formulate herbal tea which is efficacious to scavenge the free radicals. The objectives of the study are to select plants possessing antioxidant and antidiabetic properties, evaluate and compare the individual plants for their antioxidant properties with marketed formulation of green tea and to develop a polyherbal formulation with composition of herbs that has potential antioxidant activity. Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Blois[1] and Desmarchelier et al.[2] in comparison to green tea. *Aegle marmelos, Momordica charantia, Azadirachta indica, Cissus quadrangularis, Trigonella foenum graecum* were tested for antioxidant activity. *Aegle marmelos, Momordica charantia, Azadirachta indica* have shown significant Antioxidant activities. Two polyherbal tea formulations were done with compositions *Aegle marmelos : Azadirachta indica : Momordica charantia* in ratio 3:2:1(PHF1) and *Aegle marmelos : Momordica charantia : Azadirachta indica* in ratio 3:2:1(PHF2) respectively. Polyherbal formulations in fixed ratio i.e 3:2:1 of *Aegle marmelos*, *Momordica charantia*, and *Azadirachta indica* respectively showed 78% more inhibition of DPPH free radical at 20ug /ml in comparison to marketed green tea formulation. A poly herbal tea bag was prepared on weighing accurately 0.956gms *Aegle marmelos*, 0.636gms *Momordica*

*charantia* and 0.311gms *Azadirachta indica* herb powders and mixed properly and packed in a sachet. This can be used as a herbal tea for a cup containing a volume of 240ml water.

**Keywords:** DPPH, water, powders, formulations, product.

## INTRODUCTION

Tea, especially herbal tea, is attracting more consumer interest as awareness of its health benefits continues to rise. Despite the presence of numerous underutilized plants that could be processed into herbal tea, there is a scarcity of research focused on the development of such products. This study was undertaken to create an herbal tea that effectively scavenges free radicals. Oxidative stress plays a significant role in the development of numerous common health issues, affecting individuals of all age groups. Among the various diseases and conditions associated with oxidative stress are: cardiovascular diseases[3], cancer, neurodegenerative disorders[4], diabetes, liver diseases[5], and inflammatory conditions[6]. Given the extensive influence of oxidative stress, it is essential to comprehend its underlying mechanisms, contributing factors, and strategies for mitigating its detrimental effects in order to prevent and manage a variety of chronic illnesses. The health complications of oxidative stress are illustrated in Figure1. Oxidative stress is a key factor in the onset of insulin resistance, dysfunction of β-cells, and the advancement of complications associated with diabetes. The persistent presence of reactive oxygen species (ROS) in diabetic patients exacerbates blood glucose regulation and is linked to severe complications such as cardiovascular disease, neuropathy, and retinopathy. Mitigating oxidative stress through antioxidant treatments, dietary changes, and lifestyle adjustments presents considerable promise for the prevention and management of diabetes and its associated complications [8-13].

The increasing incidence of diabetes and related health conditions has sparked a heightened interest in herbal teas due to their potential preventive and therapeutic properties. Herbal teas are primarily composed of the leaves, buds, flowers, and fruits of medicinal plants, and they are recognized for their diverse biological activities, which include antioxidant, anti-inflammatory, antibacterial, anti-diabetic, and anti-cancer effects. Unlike traditional teas, which are derived from the *Camellia sinensis* plant, herbal teas consist of a variety of dried herbs, seeds, flowers, fruits, barks, and other plant components. Furthermore, tisanes are naturally free of caffeine and are often enjoyed for their appealing flavor and health benefits. Each herbal blend is typically designed to fulfill a specific function, such as promoting relaxation or mitigating the adverse effects of oxidative stress, which is crucial for the prevention and management of numerous chronic diseases. [14]. Herbal remedies have been increasingly studied for their ability to mitigate oxidative stress through their antioxidant, anti-inflammatory, and cellular-protective properties. Numerous plants contain compounds that can help neutralize reactive oxygen species (ROS), protect cellular structures, and support the body's natural detoxification and repair mechanisms. Below is a review of several commonly used herbs, supported by scientific evidence, and their role in managing oxidative stress.

Herbal teas represent a flexible and readily available means to promote health and well-being. Although scientific research validating their effectiveness is still evolving, it has been established that numerous herbs traditionally utilized in infusions possess significant medicinal qualities. Both tea in general and herbal tea specifically are attracting heightened consumer interest, driven by an increasing recognition of the health advantages associated with their consumption. Despite the existence of several underutilized plants that could be processed into herbal teas, research focused on the development of these products remains limited.

**Role of anti-oxidants**

Antioxidants can decrease the oxidative damage directly via reacting with free radicals or indirectly by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes. Antioxidants may decrease the cellular level of free radicals either by inhibiting the activities or

expressions of free radical generating enzymes such as NAD(P)H oxidase and xanthine oxidase (XO) or by enhancing the activities and expressions of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). The mechanism of action for antioxidation is represented in Figure 2.

**HERBS IN PRESENT STUDY**

***Aegle marmelos***

*Aegle marmelos* Commonly known as bael, is a tropical tree native to the Indian subcontinent. It belongs to the Rutaceae family and is widely recognized for its medicinal properties. The plant is traditionally used in Ayurvedic and folk medicine for a variety of health conditions. Reported to have anti diarrhoea, peptic ulcers healing, diuretic, analgesic, antioxidant, and antidiabetic activities, with chief chemical constituents Coumarin, Xanthotoxol, Imperatorin,Aegeline, Marmeline. [15-18].

***Azadirachta indica***

*Azadirachta indica,* commonly known as neem, is a tropical evergreen tree native to the Indian subcontinent and widely distributed throughout Asia and Africa. Neem is a member of the Meliaceae family and is renowned for its numerous medicinal properties. Often referred to as the "village pharmacy" in India, neem has been used for thousands of years in traditional medicine. Reported to have Antioxidant Activity, anticancer, anti inflammatory, wound healing effect, antidiabetic, antifungal, antimalarial, antibacterial and antiviral activities, with chief chemical constituents quercetin, nimbosterol, nimbin, kaempferol, nimbidin, Azadirachtin, Azadiradione, vepinin [19-23]

***Cissus quadrangularis***

*Cissus quadrangularis,* commonly known as Veldt Grape or Devil's Backbone, is a perennial plant belonging to the Vitaceae family. Native to tropical and subtropical regions of Africa and Asia, particularly India, this plant has been widely used in traditional medicine, especially in Ayurvedic and African healing practices. Reported to be beneficial in treatment of Diabetes, obesity, high cholesterol, bone fractures, allergies, cancer, stomach upset, painful menstrual periods, asthma, malaria, wound healing, peptic ulcer disease, weak bones, weak bones (osteoporosis) , with chief chemical constituents quercetin, Daidzein, genistein, friedelin, Vitamin 'C, quadrangularin-A, Resveratrol and piceatannol, 6-o-meta-methoxy-benzoyl catapol, Picroside and pallidol and Phytosterols. [24-27]

***Momordica charantia***

*Momordica charantia*, commonly known as bitter melon or bitter gourd, is a tropical and subtropical vine belonging to the Cucurbitaceae family. Native to Africa, Asia, and the Caribbean, this plant is widely consumed for its edible fruit, which is known for its sharp, bitter taste. Bitter melon has been used for centuries in traditional medicine systems, such as Ayurveda, Traditional Chinese Medicine (TCM), and African medicine, due to its numerous health benefits. Reported to have antidiabetic, anticancer, antioxidants, anti-inflammation, antiviral activities, and cholesterol lowering effects, with chief chemical constituents Triterpenoids, Saponins, Polypeptides, Flavonoids, Alkaloids, sterols [28-30]

***Trigonella foenum graecum***

Trigonella foenum-graecum, commonly known as fenugreek, is an annual herb in the Fabaceae (legume) family. Native to the Mediterranean region, the Middle East, and Asia, fenugreek is widely used as both a culinary spice and in traditional medicine for its numerous health benefits. Fenugreek seeds, in particular, have been the subject of significant interest due to their medicinal properties, which include anti-inflammatory, antioxidant, and antidiabetic effects. Reported to have carminative, gastric stimulant, antidiabetic, galactogogue (lactation-inducer) effects, hypocholesterolemic, antilipidemic, antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiulcer, antilithigenic activities, with chief chemical constituents Diosgenin, Trigogenin, Yamogenin, Gitogenin, Trigocoumarin, Trigoforin, Trigonelline [31-33]

***Camellia sinensis***

Camellia sinensis is the botanical name for the tea plant, the source of all traditional teas, including green tea, black tea, white tea, oolong tea, and pu-erh tea. Native to East Asia, particularly China and India, it has been cultivated for thousands of years for its leaves, which are processed in different ways to produce various types of tea. The plant is also valued for its rich chemical composition, particularly its high content of catechins and caffeine, which contribute to its numerous health benefits. Used to Treat metabolic syndrome, such as obesity, type II diabetes, and cardiovascular risk factors, with chief chemical constituents Catechins, Gallocatechins, Caffeine, Theophyline, Theobromine, Gallic acid [34-36].

**ANTIOXIDANT ACTIVITY**

Antioxidant activity can be defined as a limitation or inhibition of nutrient oxidation (especially lipids and proteins) by restraining oxidative chain reaction**s**. Oxidation is essential for many living organisms for the production of energy to fuel biological processes however ,the uncontrolled production of oxygen derived free radicals is involved in triggering many diseases such as cancer , rheumatoid arthritis , cirrhosis and atherosclerosis as well as in degenerative processes associated with ageing. The aim of this work is to conduct evaluation of antioxidant properties of selected polyherbal formulation extracts.

**Principle**

**DPPH Radical Reducing Activity:**

DPPH is a cell membrane stable free radical that acts as a hydrogen radical scavenger. The DPPH radicals are widely used to investigate the scavenging activity of natural compounds. In DPPH assay it involves reduction of alcoholic DPPH by using a free radical of DPPH (1,1 -Diphenyl -1-picrylhydrazyl). The odd electron in the DPPH free radical gives a strong absorption at 517 nm and is purple in colour. The colour turns from purple to yellow when an odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced (Diphenyl -picrylhydrazine) DPPH-H. The resulting decolourisation is stoichiometric with respect to the number of electrons captured Figure3.

**Preparation of hot infusion:**

*Aegle marmelos, Azardirecta indica, Momordica charantia, Cissus quardangularis , Trigonella foenum graceum* plant fresh leaves are collected, washed properly, shade dried and are pulverised**.** These powdered leaves are taken and weighed 0.1g each and added to 100ml of the hot boiling water separately and infused for 1minutes. In the same way the marketed *Camellia sinensis* is infused for comparison of the marketed antioxidant activity with these selected *Aegle marmelos, Azardirecta indica , Momordica charantia, Cissus quardangularis , Trigonella foenum graecum* plants. Plan of work is showed in figure 4.

**Preparation of 0.004% of methanolic DPPH Solution:**

6 mg of 1,1 -Diphenyl -1-picrylhydrazyl is accurately weighted and dissolved in 150 ml of methanol to make methanolic DPPH solution.

**Preparation of dilutions**

The hot infusions are filtered with Whatman filter paper and from the filtrate 10 ml is taken and the volume is made up to 100 ml with distilled water which makes it 100ug/ml. From these hot infusions various concentrations like 20, 40, 60 ,80 and100 µg /ml are taken i.e. by taking in various concentrations as in Table 1.

Now 1 ml of each plant extract is taken and 3 ml of Methanolic DPPH solution is added to the test tube and covered with aluminium foil and kept in a dark place (as DPPH is light sensitive) to react for **1 hour.**

## RESULTS

The reacted plant extract with Methanolic DPPH solution is checked for the absorbance at 517ŋm in UV-visible spectrophotometer of make Elico double beam Sl.210 using ethanol as blank and ethanolic DPPH solution as control

**Calculation of Percentage inhibition:**
The percentage inhibition of DPPH radical production by the extract was calculated using the formula:

$$Inhibitory\ Ration = \frac{(A_0 - A_1)X\ 100}{A_0}$$

Where , $A_o$ is the absorbance of control;
$A_1$ is the absorbance with addition of plant extract

The absorbance, transmission and percentage inhibition of DPPH radical for various plant extracts is tabulated in Tables 2-7. The absorbance were measured at 517nm using ethanol as blank and ethanolic DPPH solution as control. The comparison of anti-oxidant activities were done in Figure 5.

- Ethanol:- 0.0882 absorbance
- Ethanoic DPPH solution: 1.4227 absorbance

**Preparing polyherbal mixture in ratios based on % inhibition values:**
Based on the % inhibition values of individual plants in comparison to green tea, 3 plant samples were selected they are

1. *Aegle marmelos*
2. *Azadirachta indica*
3. *Momordica charantia*

They were weighed in two different ratios which are

1. *Aegle marmelos:Azadirachta indica:Momordica charantia* (3:2:1) as 300mg ,200mg and 100mg respectively they are mixed well and 100mg of the mixture is weighed and infused with 100ml boiled water for 10minutes.
2. *Aegle marmelos: Momordica charantia: Azadirachta indica* (3:2:1) as 300mg ,200mg and 100mg respectively are mixed well and 100mg of mixture is weighed and infused with 100ml boiled water for 10minutes.

Later both the combinations are filtered and from the filtrate and 10 ml of filtrate is diluted to 100ml with distilled water to make it 100µg/ml. From the above Concentration 10ml, 8ml, 6ml, 4ml, 2ml are taken and diluted to 10ml with distilled water making the 100µg , 80µg ,60µg , 40µg , 20µg per ml concentrations. These were measured for absorbance at 517 nm in UV-Visible spectrophotometer. Green tea hot infusion is also prepared for 1mg/ml concentration and series dilutions of 100µg, 80µg, 60µg, 40µg, 20µg per ml concentrations and absorbance is measured at 517 nm in UV visible spectrophotometer taking Ethanol as blank and Ethanolic DPPH as control.

**Sachets preparation**
0.956gms *Aegle marmelos*, 0.636gms *Momordica charantia* and 0.311gms *Azadirachta indica* powders are weighed accurately and mixed properly and packed in a sachet. This can be used as a herbal tea for a cup containing a volume of 200ml

**Comparison of green tea with Polyherbal Formulations**

The prepared poly herbal formulations were compared with commercial Green tea sachet, hot infusions were prepared and made to react with Ethanolic DPPH solution is checked for the absorbance at 517ŋm in UV-visible spectrophotometer of make Elico double beam Sl.210 using ethanol as blank and ethanolic DPPH solution as control

The absorbance, transmission and percentage inhibition of DPPH radical for poly herbal formulation 1(PHF1) and poly herbal formulation 2(PHF2) in comaprision with marketed green tea formulation is tabulated in **Tables 8-10.** The absorbance were measured at 517nm using ethanol as blank and ethanolic DPPH solution as control. The comparison of anti-oxidant activities for PHF1, PHF2 and green tea were done in F**igure 6.**

- Ethanol 0.0001 absorbance and 99.98 transmittance
- Ethanolic DPPH solution 0.4884 absorbance and 32.48 Transmittance

## SUMMARY AND CONCLUSION

All the five plants *Aegle marmelos, Momordica charantia, Azadirachta indica, Cissus quadrangularis, Trigonella foenum graecum* have shown Antioxidant properties in comparison to marketed formulation Green tea. The concentrations at which individually plants showed highest activity at 100ug/ml concentration Aegle marmelos showed 53.18%, Azadirachta indica showed 60.63%, at 80ug/ml concentration Momordica charantia showed 40.78%, at 60 ug /ml concentration cissus quadrangularis showed 38.28 %, 20ug/ml Trigonella foenum graecum showed 35.23% and at 100ug/ml green tea showed 55.37%. Wherein polyherbal formulations in fixed ratio i.e 3:2:1 of Aegle marmelos, Momordica charantia, and Azadirachta indica respectively showed 78% more inhibition of DPPH free radical at 20ug /ml in comparison to marketed green tea formulation.

**Future Scope**

The future scope of the project is development of a polyherbal antidiabetic formulation based on the results obtained in the In-vitro antioxidant tests performed on the plants Aegle marmelos, Azadirachta indica , Cissus quadrangularis , Momordica charantia ,Trigonella foenum graecum in comparison to marketed Camelia sinensis individually and in combination of Aegle marmelos, Azadirachta indica and Momordica charantia performed for the plants selected as these plants show the antioxidant properties are said to have the antidiabetic property as per the literature so this formulation can further be screened for the antidiabetic property.

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**Table 1: preparation of infusions with different concentration**

| Concentration in micrograms | Plant exact taken (ml) | Water (ml) |
|---|---|---|
| 20 µg /ml | 2 | 8 |
| 40 µg /ml | 4 | 6 |
| 60 µg /ml | 6 | 4 |
| 80 µg /ml | 8 | 2 |
| 100 µg /ml | 10 | - |

**Table 2: Antioxidant activity of Green tea in Ethanolic DPPH solution**

| Green tea | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **% inhibition** |
| 20 | 1.114 | 7.69 | 21.69 |
| 40 | 1.0615 | 8.67 | 25.38 |
| 60 | 0.923 | 11.94 | 35.12 |
| 80 | 0.824 | 15.09 | 42.28 |
| 100 | 0.6349 | 23.18 | 55.37 |

**Table 3: Antioxidant activity of *Aegle marmelos***

| *Aegle marmelos* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.7821 | 16.13 | 45.02 |
| 40 | 0.7576 | 17.47 | 46.74 |
| 60 | 0.7167 | 19.2 | 49.62 |
| 80 | 0.699 | 21.42 | 52.97 |
| 100 | 0.666 | 21.58 | 53.18 |

**Table 4: Antioxidant activity of *Azardirachta indica***

| *Azardirachta indica* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.6197 | 24 | 56.44 |
| 40 | 0.6585 | 21.94 | 53.69 |
| 60 | 0.6975 | 20.07 | 50.97 |
| 80 | 0.6101 | 25.54 | 57.116 |
| 100 | 0.5601 | 27.54 | 60.63 |

**Table 5: Antioxidant activity of *Momordica charantia***

| *Momordica charantia* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.8642 | 13.14 | 39.25 |
| 40 | 0.8544 | 13.96 | 39.94 |
| 60 | 0.8448 | 14.52 | 40.619 |
| 80 | 0.8425 | 14.82 | 40.78 |
| 100 | 0.8585 | 13.84 | 39.656 |

**Table 6: Antioxidant activity of *Cissus quadrangularis***

| *Cissus quadrangularis* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.9032 | 12.5 | 36.51 |
| 40 | 0.896 | 12.7 | 37.02 |
| 60 | 0.9207 | 12 | 38.28 |
| 80 | 0.8943 | 12.75 | 37.14 |
| 100 | 0.8891 | 12.9 | 37.15 |

**Table 7: Antioxidant activity of *Trigonella foenum graecum***

| *Trigonella foenum graecum* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.9214 | 11.98 | 35.23 |
| 40 | 0.9314 | 11.71 | 34.53 |
| 60 | 0.9832 | 10.39 | 30.891 |
| 80 | 0.9359 | 10.59 | 34.216 |
| 100 | 0.9916 | 10.19 | 30.301 |

**Table 8: Antioxidant activity of Green tea in Ethanolic DPPH solution**

| *Green tea* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.3220 | 47.75 | 34.07 |
| 40 | 0.2839 | 51.93 | 41.87 |
| 60 | 0.2234 | 59.79 | 54.25 |
| 80 | 0.2203 | 60.21 | 54.89 |
| 100 | 0.1812 | 65.89 | 62.89 |

**Table 9: Antioxidant activity of PHF1 in Ethanolic DPPH solution**

| *Aegle marmelos:Azadirachta indica:Momordica charantia(3:2:1)* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.2940 | 50.82 | 39.80 |
| 40 | 0.2575 | 55.27 | 47.27 |
| 60 | 0.2305 | 58.82 | 52.80 |
| 80 | 0.2579 | 55.22 | 47.19 |
| 100 | 0.2766 | 52.89 | 43.36 |

**Table 10: Antioxidant activity of PHF2 in Ethanolic DPPH solution**

| *Aegle marmelos : Momordica charantia: Azadirachta indica (3:2:1)* | | | |
|---|---|---|---|
| **Concentration µg/ml** | **Absorbance** | **Transmittance** | **%inhibition** |
| 20 | 0.1916 | 64.33 | 60.76 |
| 40 | 0.2259 | 59.44 | 53.74 |
| 60 | 0.1767 | 66.57 | 63.82 |
| 80 | 0.1576 | 69.57 | 67.73 |
| 100 | 0.2954 | 50.65 | 39.516 |

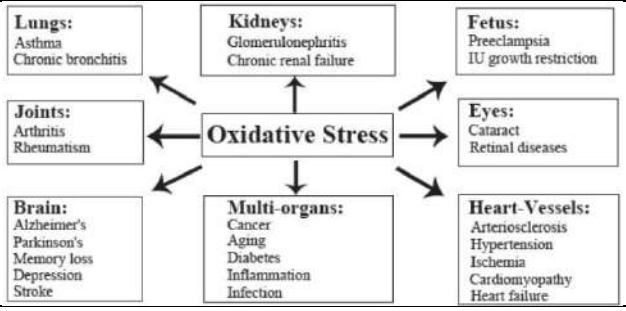


**Figure 1: Health Complications of oxidative stress Pham-Huy LA, et al. He H, Pham-Huy C., et al [7]**

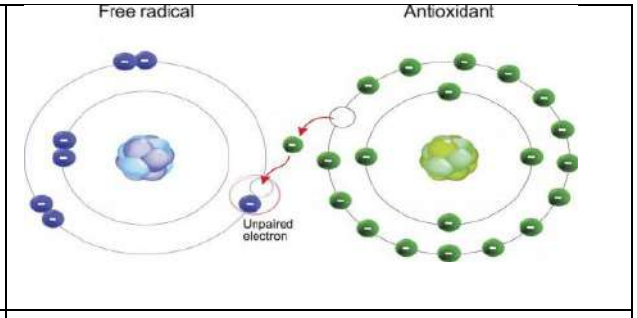


**Figure 2: Antioxidants Mechanism of action Zawn Villines et al.**

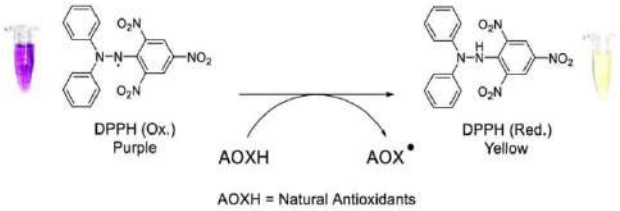


**Figure 3: Principle involved in DPPH Radical Reducing Activity**

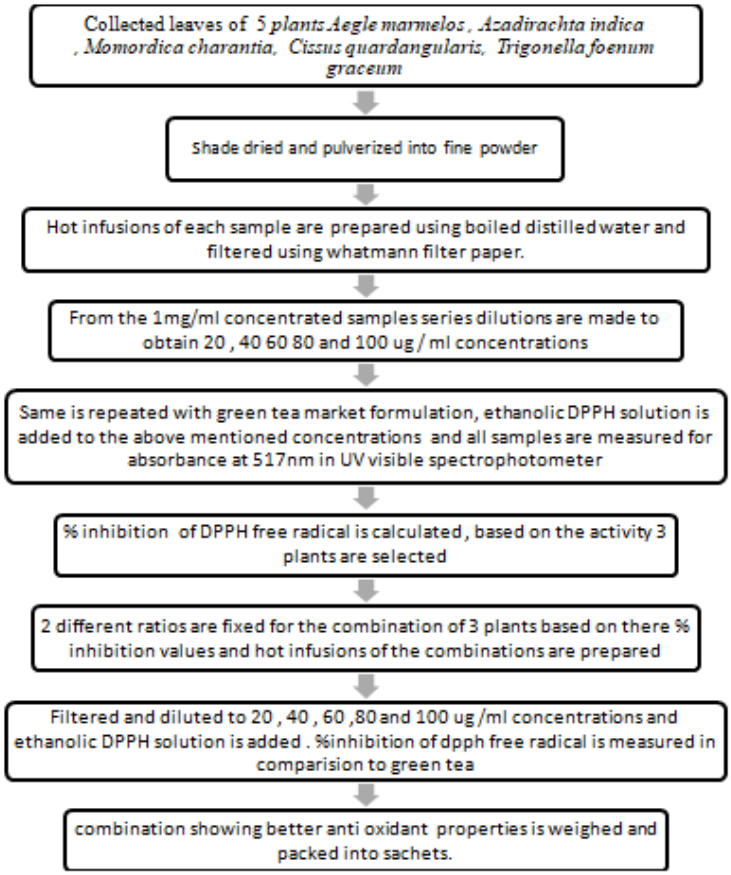


**Figure 4: Plan of work**

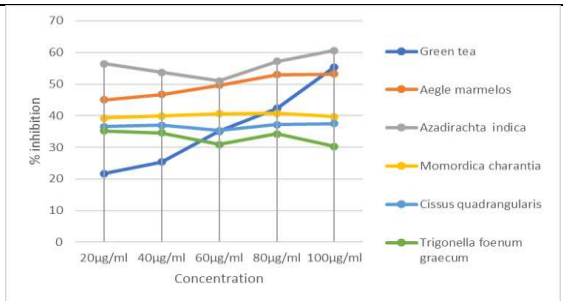


**Figure 5: Comparison of antioxidant Activities**

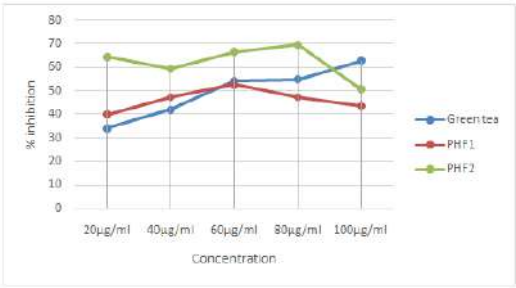


**Figure 6: comparison of Antioxidant activity of PHFs with Green Tea in Ethanolic DPPH solution**

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**RESEARCH ARTICLE**

# Molecular Docking Investigation into the Hepatic Effects of Cyclooxygenase and CYP2E1 by the Methanol Extract of *Padina gymnospora* in Male Albino Wistar Rats

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## ABSTRACT

Abstract: Proteins are vital macronutrients essential numerous functions for sustaining all living organisms, as they possess the unique ability to bind various molecules. This study kingpin on the methanolic extract of the *Padina gymnospora* is a brown seaweed, which yields several important fatty acids including Stearidonic acid, 9-Oxo-10,12-octadecadienoic acid, Nonadecanoic acid, Oleic acid, Linoleic acid, Eicosanolic acid, Arachidonic acid, Eicosapentaenoleic acid, Linolenic acid, and Palmitoleic acid. Among these components, Nonadecanoic acid stands out with a negative charge (-0.07). Paracetamol, a widely used analgesic and antipyretic drug, undergoes oxidation primarily through the enzyme CYP2E1 (Cytochrome P450 2E1). Besides CYP2E1enzyme, its subclasses CYP1, CYP2, and CYP3, facilitates the breakdown of foreign compounds in mammals. Although Paracetamol does not belong to the NSAIDs family, it acts on cyclooxygenase (COX) isoenzymes COX-1 and COX-2, by inhibiting prostaglandin H (PGHS) production. PGHS, with its dual functionality as cyclooxygenase and peroxidase, plays a crucial role in inflammation. Paracetamol exerts its pain-relieving effects by modulating tissue receptors in the spinal cord, thalamus, and cerebral cortex, where pain sensations are processed. Additionally, it induces the production of fatty acid amide N-arachidonoylphenolamine (AM404) through the L-arginine/NO pathway and descending serotoninergic routes within the brain. Molecular modeling techniques analysis shows that both the 4th position phenolic OH group of silymarine and Paracetamol bind to the carboxylic acid of Nonadecanoic acid in *Padina gymnospora*. This interaction highlights a potential mechanism for the therapeutic action of Paracetamol in association

with fatty acids from seaweed extracts. Graphical abstract the hepatoprotective of *Padina gymnospora* is shown in Figure:1.

**Keywords:** Protein, paracetamol, silymarine, nonadecanoic acid, cyclooxygenase, Cytochrome P450, L-arginine pathway, serotoninergic pathway.

## INTRODUCTION

*Padina gymnospora* was collected from Gulf of Mannar area, Tuticorin located (8.76°42″ N; 78.13° 48″ E) at Rameswaram, district Ramanathapuram in Tamil Nadu. Phaeophyta (brown algae) are more abundant in a shallow rocky, low tide area. The plant was identified and authenticated at the herbarium (AU Bota#609) of Botany, Professor Dr.L.Mullainathan , Academic affairs Director, Faculty of Science, Annamalai University, Tamilnadu, India. *Padina gymnospora* [(Kützing) Sonder 1871)**]** thallus (Figure 2B) of *Padina gymnospora* is medium brown on the upper surface and dark brown below, with a relatively robust structure. It is often lightly calcified on the upper surface and measures 5–15 cm in length and 5–20 cm in spread. When young, it has a flabellate shape, becoming shallowly to deeply divided with cuneate to flabellate segments that are mostly 1–5 cm wide at the top, featuring acute to rounded axils. The thallus is attached to substrates by a rhizoidal holdfast measuring 0.5–2 cm in length and 0.2–1 cm in width, making it epilithic [Figure:2(A)] Paracetamol, Silymarin and Nonadecanoic acid from *Padina gymnospora* are three ligands and COX-2 and p450 are two proteins who are binding with them.

**Paracetamol:** (Molecular formula $C_8H_9NO_2$ ) is a dependence, tolerance, safe antipyretic or analgesia drug without risk factoring when it is used alone. Using of large quantity of paracetamol is dangerous harmful of hepatotoxicity. Paracetamol undergoes metabolic changes, resulting in the creation of two main compounds: N-(4-hydroxyphenyl)-arachidonamide (AM404) catalyzed by fatty acid amide hydrolase (FAAH) and N-acetyl-p-benzoquinone imine (NAPQI) facilitated by cytochrome P450 (CYP) enzymes. Despite these known metabolic pathways, the fundamental mechanism underlying the actions of paracetamol remains unclear. Additionally, paracetamol exhibits interactions with drugs involving the CYP family enzymes, such as inducers, inhibitors, and substrates, including illicit drugs. In contrast to ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), The uncertainty persists about whether analgesic effect of paracetamol stems from the inhibition of cyclooxygenases (COXs). [1]. A crucial metabolic pathway of significance involves the hepatic cytochrome P450-dependent mixed-function oxidase system converting paracetamol to a reactive metabolite [2,3]. This active metabolite is hypothesized to be either N-acetyl-para-benzoquinoneimine [4] or its related semiquinone free radical form [5,6]. Paracetamol induced hepatotoxicity are balanced both 'toxic' and 'detoxification' pathway. A similar relationship was observed in rats, where the covalent binding of radiolabel to liver proteins 48 hours after administering [ring-14C]-paracetamol was directly proportional to the extent of liver damage [7]. It showed the evidence of covalent binding of radiolabel to liver plasma membranes and microsomes 2.5 hours after oral administering 2.5 g/kg body weight of $^3$H-paracetamol to rats [8].

**Silymarin:** (Molecular formula $C_{25}H_{22}O_{10}$.) is categorized as a hepatoprotective medication, designed to safeguard the liver. For centuries, milk thistle extracts are recognized as beneficial for the liver, often referred to as "liver tonics." These extracts are reported to possess protective properties that enhance liver function. Milk thistle is frequently used to address ailments like liver cirrhosis, chronic hepatitis (liver inflammation), toxin-induced liver damage (including protection against severe effects from Amanita phalloides, the 'death cap' mushroom poisoning), and disorders related to the gallbladder. In a specific study, individuals with Type II Diabetes experienced noteworthy reductions in Hemoglobin A1C levels, and fasting blood sugar , insulin, liver enzymes SGOT or AST and SGPT or ALT, LDH after the administration of silymarin at a dosage of 200 mg thrice daily. Additionally, supplementation with silymarin showed notable improvements in specific antioxidant indicators (total antioxidant capacity and thiol levels) and a reduction in liver enzyme levels in patients suffering from trauma-induced liver injury. It is noteworthy that the two species naturally interbreed, giving rise to a hybrid known as Silybum × gonzaloi Cantó, Sánchez Mata & Rivas Mart. (a cross between *S. eburneum* var. hispanicum and *S.marianum*) [9]. Different constituents of milk thistle

(such as silymarin, silybin, etc.) possess diverse mechanisms of action that could potentially safeguard the liver, including anti-inflammatory properties, antioxidant effects, blocking toxins, promoting protein synthesis, and anti-fibrotic effects [10,11].

Silymarin facilitates the transport of bile acid molecules from the liver to the small intestine and back to the liver. The term "enterohepatic circulation" (EHC) describes the complex process wherein bile acid molecules travel from the liver to the small intestine and then return to the liver. This cyclical movement entails the active secretion of bile acids into canalicular bile within hepatocytes, thus completing the enterohepatic cycle. During this circulation, bile salts interact with facultative and anaerobic bacterial populations, which, though relatively sparse in number, display substantial diversity in the small bowel.

**Nonadecanoic acid:** Nonadecanoic acid (Molecular formula $C_{19}H_{38}O_2$ / $CH_3(CH_2)_{17}COOH$) is an odd chain (19 carbon) saturated fatty acid which is primarily produced by initiating the synthetic series with the three-carbon compound, propionic acid. Propionate must be converted to succinate in order for the primary energy pathway to be oxidized, and this requires vitamin $B_{12}$. *Padina gymnospora*, a type of dark brown algae from the Dictyotacaea family, exhibits a distinctive morphology with its base and fan-shaped lobes dividing into wedge-shaped pieces. This algae is well recognized for playing a significant role in the healing of wounds naturally [12]. Moreover, it demonstrates notable medicinal properties, serving as an agent against cancer, bacteria, inflammation, and oxidative stress [14], while also showing larvicidal activity [13]. To detect the molecules within the methyl alcohol extract of P. gymnospora being investigated, a solution was prepared by dissolving 10 μL of the methyl alcohol extract in a 1000 μL mixture of methanol and water (50% v/v). The electrospray ionization (ESI-) negative ion mode of the mass spectrometer employed for the analysis covered a mass range of m/z 150 to 1500 [14]. FTIR (Fourier Transform Infrared Spectroscopy) utilizes infrared absorption spectra to discern the chemical bonding within molecules, facilitating the screening and scanning of samples for various components. On the other hand, SEM (Scanning Electron Microscopy) generates high-resolution images for the detailed analysis of a sample's chemical characteristics.

**COX-2 (Cyclooxygenase-2):** Prostaglandin-endoperoxide synthase (PTGS), commonly referred to as cyclooxygenase, is essential for the synthesis of prostaglandins such prostacyclin and prostanoids like thromboxane which come from arachidonic acid, a polyunsaturated omega-6 fatty acid characterized by a 20-carbon chain and four cis-double bonds, with the first double bond positioned at the sixth carbon from the omega end. Phospholipids, specifically phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides, are composed of polyunsaturated fatty acids and are responsible for the membranes of body cells. They are notably abundant in the brain, muscles, and liver. Inhibition of COX through pharmaceutical means can alleviate symptoms of inflammation and pain [15]. Nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and ibuprofen work by inhibiting COX. Drugs that target specifically the COX-2 isozyme are referred to as COX-2 inhibitors. Paracetamol's active metabolite (AM404) also acts as a COX inhibitor, a characteristic that contributes to its therapeutic effects, potentially involving G-protein coupled membrane receptors [ 16]. Paracetamol's mechanism of action includes the inhibition of cyclooxygenases (COX-1, COX-2, and COX-3) and involvement in the endocannabinoid system and serotonergic pathways. Additionally, it impacts transient receptor potential (TRP) channels and voltage-gated Kv7 potassium channels, while also inhibiting T-type Cav3.2 calcium channels. Paracetamol additionally modulates the L-arginine pathway in nitric oxide (NO) synthesis. Cyclooxygenase activity was assessed using an oxygen electrode with 100μM arachidonic acid as the substrate [17], while inhibition studies involving IBP were conducted [18]. The thermal stability of protein in different chemical environments (Thermal shift assays) were performed using apo muCOX-2 and a Stratagene Mx3005P real-time PCR instrument [19]. Coordinates and the fundamental quantities on which the function of electron density depends on the Protein Data Bank (PDB ID: 4PH9) [20].

**Cytochrome P450:** Cytochrome P450 (CYP) enzymes, membrane-bound hemoproteins, are pivotal in detoxifying xenobiotics, regulating cellular metabolism, and upholding homeostasis. The induction or inhibition of these enzymes stands as a significant mechanism driving drug-drug interactions. Different xenobiotics and endogenous

substrates have the potential to activate CYP enzymes transcriptionally via receptor-dependent mechanisms. Inhibition of these enzymes serves as a fundamental mechanism for metabolism-related drug-drug interactions. Chemotherapeutic medications, in particular, can precipitate such interactions by either inhibiting or inducing the CYP enzyme system. Through in silico analyses, followed by validation, many short RNA molecules have been found in eukaryotic cell as key regulators of CYPs. Furthermore, genetic polymorphisms and epigenetic alterations in CYP genes may subscribe to variances in disease susceptibility and the remedial efficacy of drugs among individuals and across different ethnicities. The super family of hemoproteins known as cytochromes P450 (CYP P450s) in the liver of humans is in charge of catalyzing the oxidative metabolism of medications and foreign substances when they enter the body. Adverse events and treatment failures are frequently caused by interactions between medicines or xenobiotics. When inducers are used at the same time with other CYP450-metabolizing medications, changes in their metabolism may occur in the liver or gastrointestinal tract. The therapeutic intention of these interactions is related to autoinduction, which has the potential to cause a certain result therapy outcomes that are not ideal or successful. Pharmaceutical corporations have worry about the situation of matter when developing new medications.

Non-CYP-mediated oxidative processes are equally important for xenobiotic metabolism, even though the CYP is a group of homologous enzymes that share a common mechanistic attribute but catalyze different overall reactions, mainly mediates oxidative metabolic reactions. Together with CYPs, these non-CYP oxidase include lipoxygenases, prostaglandin H synthase, flavin-containing monooxygenases, alcohol and aldehyde dehydrogenases, molybdenum hydroxylases (like aldehyde oxidase and xanthine oxidase), amine oxidases (like monoamine, polyamine, diamine, and semicarbazide-sensitive amine oxidases), and lipoxygenases. Like CYPs, these catalyze oxidation reduction reactions can produce reactive/toxic metabolites in addition therapeutically active metabolites, which can affect the effectiveness of remedial agent medications or aid in detoxification. Numerous them are important players in the metabolism of endobiotics, which may result in interactions between medications and endogenous molecules when pharmaceuticals are being metabolized. Within the enzyme CYP2E1 (Cytochrome P450 2E1), there are five proteins: 3GPH, 3KOH, 3T3Z, 3LC4, and 3E4E. [ Figure:2(B)]

## MATERIALS AND METHODS

*Padina gymnospora* extracts both Soxhlet and filtration process:- The choice of solvent for soxhlet extraction is crucial and typically based on polarity and the phytoconstituent isolation process. It's important for the solvent to be easily removable and inert. Methanol, being a semi-polar solvent, is often preferred as it can effectively extract many phytoconstituents

**Experimental Design**

Albino rats were segregated into seven groups, each consisting of six individuals:

**Group 1** (Control): Administered physiological saline solution at a dosage of 10ml/kg body weight.

**Group 2:** Received a single dose of paracetamol at 3 g/kg weight of the body via oral gavage (Size 16 Gauge, Length 3 inches, Ball diameter 3mm).

**Group 3:** Given paracetamol along with *Padina gymnospora* at a dosage of 50 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.

**Group 4:** Given paracetamol along with *Padina gymnospora* at a dosage of 100 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.

**Group 5:** Given paracetamol along with *Padina gymnospora* at a dosage of 200 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.

**Group 6:** Given paracetamol along with silymarin at a dosage of 25 mg/kg weight of the body daily one time for 28 days, administered orally using the same oral gavage method as Group 2.

**Group 7:** Administered *Padina gymnospora* alone at a dosage of 200 mg/kg weight of the body daily one time for 28 days, using the same oral gavage method as Group 2.

The animals were euthanized by cervical dislocation 24 hours after the final dose, marking the end of the tentative test. Blood specimens were obtained without the use of an anticoagulant to separate serum and measure serum marker enzymes such as AST, ALT, ALP, LDH, GGT, bilirubin, cholesterol, and protein levels. The liver tissue was instead of taken away, washed with cold physiological saline, and preserved in 10% formalin for subsequent histological analyses.

**UniProt Database:** The UniProt Knowledgebase (UniProtKB) acts as the principal repository for extensive protein data, providing accurate, standardized, and detailed annotations. The FASTA sequence was retrieved from the UniProt database, with the UniProt ID assigned as Q99497. Additionally, the corresponding PDB ID was sourced from the UniProt database. Protein-ligand binding denotes the interaction wherein a small molecule, frequently a drug (ligand), attaches to a receptor or protein within the body, triggering a biological response such as inflammation reduction or pain relief. The hurdle in drug discovery lies in pinpointing the precise bioactive pose of the protein-ligand complex, which may adopt a restricted number of poses or configurations.
Determining the bioactive pose is complex since there isn't always a sole lowest energy pose for either the protein or the ligand. The complex might stabilize a higher energy conformation of the ligand. Both the protein and ligand are three-dimensional and flexible, constantly altering their shape. This presents a multi-step challenge, beginning with identifying the bioactive 3D conformation of the ligand. As we shift to the protein, this always daunting due to its larger size and expanded possibilities.

Successfully pinpointing both the bioactive conformation of the ligand and the protein presents the additional hurdle of accurately positioning the ligand within the protein to attain the desired activity. Various methods are available to generate these poses, differing in computational expense. One approach entails employing a cost-effective method to produce a vast array of potential poses, succeeded by a more resource-intensive calculation to prioritize them based on the probability of being the bioactive pose. This process aims to generate promising binding configurations for further study. The Protein Data Bank (PDB) serves as an official experimental repository housing three-dimensional structures of proteins, nucleic acids, and other biological macromolecules. The PDB format, denoted by the .pdb extension, comprises a file format containing a four-character alphanumeric identifier (e.g., 3T3Z), which aids in the description and annotation of protein and nucleic acid structures, including atomic coordinates and connectivity. Additionally, the PDB format encompasses information relevant to structural biology, such as gene expression, in-silico methodologies, and studies on protein-nucleic acid interactions. This standard format is used for structures deposited in the Protein Data Bank (PDB) at the Research Collaboratory for Structural Bioinformatics (RCSB) [21] .

**Molecular docking studies:** The Lamarckian genetic algorithm was utilized in conducting investigations on flexible ligand docking [22]. CB-Dock2 stands as an improved iteration of the CB-Dock server, specifically designed for protein-ligand blind docking. This upgraded version seamlessly integrates features for cavity detection, docking, and homologous template fitting. Through the utilization of the protein and ligand's three-dimensional (3D) structures, CB-Dock2 facilitates the prediction of binding sites and assesses affinity, contributing and the power to produce a desired result. of computer-aided drug discovery endeavors. ChemBioDraw Ultra 12.0 was used to create the ligand molecules initially, and ChemBio3D Ultra 12.0 was used to transform them into their three-dimensional structures. After that, energy conservation was done with the MOPAC Ultra 2009 program utilizing the PM3 technique [23].The resultant ligands, post-preparation, were utilized as input files for the CB Dock 2 online software (**see figure-3**). CB-Dock 2 performs an initial retrieval from our server's protein-ligand complexes database. This retrieval seeks template ligands with high topology similarity (FP2 ≥ 0.4). If such templates are found, CB-Dock2 calculates the similarity between the user's protein and proteins complexed with the selected template ligands. Retained are complexes with over 40% sequence identity and a pocket RMSD of ≤ 4Å in the template ligand binding site. These selected complexes then undergo template-based cavity detection followed by molecular docking. The docking process utilizes FitDock, an internally developed technique that employs a hierarchical multi-feature alignment approach to align the initial conformation with the specified template. It then explores potential conformations and produces refined docking poses. Upon file submission, an automated Perl script will handle the processing of the required files. The script utilizes the most recent release (version 1.2.0) of AutoDock Vina, specifically designed for

template-independent blind docking. The blind docking pipeline follows a template-based approach and incorporates the BioLip database (as of September 15, 2021) as the template repository for guiding the docking process.

The default worth for all other parameters were maintained. After docking, hydrogen bonding and π–π interactions were examined in the top poses, and the root mean square deviation (RMSD) was computed using Discovery Studio Visualizer 2.5. Each ligand's inhibition constant (Ki) was calculated using the predicted free energy of its binding (ΔGbinding, kcal/mol). The model with the lowest Discrete Optimized Protein Energy (DOPE) score was selected, refined, and evaluated using structural characteristics such as the Ramachandran plot with PROCHECK [24], the ERRAT plot [25], and the ProSA [26] web servers. Additionally, the model underwent energy minimization before being used in docking and molecular dynamics (MD) simulation studies.

**Physicochemical parameters Calculation:** Absorption (%ABS) was calculated using the formula: %ABS = 109 – [0.345 × topological polar surface area (TPSA)], as described by Zhao et al [27]. TPSA [28], miLogP, the number of rotatable bonds, and adherence to Lipinski's "Rule of Five" [29] were determined using the Molinspiration online property calculation toolkit [30]. The molecular binding are shown in the below Table-A.

## RESULTS AND DISCUSSION

Bold and under line docking works (binding energy and related amino acids) which are shown the Table-A and Table:1 to Table:6 with binding images are provided in below (Results and Discussion).Also Graphical represented of molecular binding (protein ligand bond).

Milk thistle, particularly silymarin, exhibits hepatoprotective effects against liver cirrhosis, viral hepatitis, and alcoholic liver disorders. In an experiment involving Paracetamol combined with *Padina gymnospora* (200mg/body weight), liver enzyme levels of AST, ALT, and LDH were measured at 165mg/dL, 145.5mg/dL, and 131mg/dL respectively. When *Padina gymnospora* was combined with silymarin at 25mg/Kg body weight, these enzyme levels decreased to 123mg/dL, 137.9mg/dL, and 146mg/dL respectively, indicating a reduction in AST, ALT, and LDH levels with silymarin use. It has observed that Paracetamol, silymarin, and nonadecanoic acid from *Padina gymnospora* inhibit cytochrome P-450 and Cyclooxygenase 2 (COX-2). When *Padina gymnospora* was administered alone at 200mg/Kg body weight, AST, ALT, and LDH values were 170mg/dL, 479mg/dL, and 128mg/dL respectively. This suggests a weaker interaction (loose binding of -3.62) with COX-2. However, when Paracetamol and silymarin were combined, the binding energy improved significantly (-5.5) (See Graphical represented molecular binding (protein ligand bond).

The binding affinities of these compounds with specific proteins were also examined. COX-2 (PDB id-4PH9) exhibited binding with nonadecanoic acid (-6.1), paracetamol (-6.3), and silymarin (-10.2). In comparison, CYP2E1 (Cytochrome P4502E1) displayed different protein binding characteristics, with nonadecanoic acid showing good binding to protein 3GPH (-8.1), paracetamol to 3LC4 protein (-7), and silymarin to 3T3Z (-10.8). Overall, the research indicates that P-450 binding is more significant than COX-2 binding. The presence of nonadecanoic acid with paracetamol appears to strongly bind and potentially treat liver diseases induced by paracetamol toxicity. The study explores the potential therapeutic effects of *Padina gymnospora*, a type of brown seaweed, focusing on its methanolic extract rich in various fatty acids, notably Nonadecanoic acid. Paracetamol, a commonly used analgesic, is metabolized by the enzyme CYP2E1 and affects cyclooxygenase isoenzymes, modulating pain sensation and inducing the production of AM404. Molecular docking analysis suggests a potential interaction between Paracetamol and silymarin with Nonadecanoic acid, shedding light on a possible mechanism underlying the therapeutic action of Paracetamol in conjunction with fatty acids from seaweed extracts.

## CONCLUSION

The investigation focused on the binding pockets of COX-2 and Cytochrome P450 2E1 as part of the quest for small molecules with potential in combating liver disease. Through a comparative analysis, it was determined that the silymarin ligand exhibited a higher docking score compared to remdesivir and displayed superior binding affinity compared to remdesivir in these contexts. Nonadecanoic acid is better binding with cytochrome P450 than Cyclooxygenase-2 due to its' ability of high binding .

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**Conflict of interest**
Regarding the content of this article, the writers declare that they have no conflicts of interest.

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**Author's contribution**
In addition to producing the initial draft of the manuscript, the author also assisted with its conceptualization, data curation, formal analysis, funding procurement, research, methodology, resource management, software development, supervision, validation, and visualization.

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**Table - A: Table of Molecular Binding**

| PDB ID | Molecular Name Protein | Nonadecanoic acid | Paracetamol | Silymarin |
|---|---|---|---|---|
| 4PH9 | The configuration of Ibuprofen attached to cyclooxygenase-2[31] | -6.1 | **-6.3** | **-10.2** |
| 3GPH | The human cytochrome P450 2E1 complexed with omega-imidazolyl-decanoic acid[32] | **-8.1** | -6.8 | -8.6 |
| 3KOH | Cytochrome P450 2E1 in complex with omega-imidazolyl octanoic acid.[32] | -7.4 | -6.7 | -10.7 |
| 3T3Z | The human Cytochrome P450 2E1 bound with pilocarpine[33] | -7.1 | -6.2 | **-10.8** |
| 3LC4 | The complex of human Cytochrome P450 2E1 with Omega-Imidazolyl-Dodecanoic Acid[32] | -7.5 | **-7** | -10.1 |
| 3E4E | The human cytochrome P450 2E1 bound to the inhibitor 4-methylpyrazole[34] | -6.6 | -5.9 | -9.6 |

**Table no-1 Nonadecanoicacid with COX-2 (4PH9 protein) binding energy and related aminoacids:-**

| Ligand Name | PDB Code | Amino acid residue | Nature of Interaction | Binding Distance(Å) | Binding Energy (kcal/mol) |
|---|---|---|---|---|---|
| Nonadecanoic acid | 4PH9 | TYR A:386 | Conventional Hydrogen Bond | 2.58 | -6.1 |
| | | THR A:207 | Conventional Hydrogen Bond | 2.71 | |
| | | ALA A:203 | Alkyl | 5.02 | |
| | | ALA A:200 | Alkyl | 4.85 | |
| | | LEU A:391 | Alkyl | 4.76 | |
| | | LEU A:392 | Alkyl | 5.29, 4.02, 4.46 | |
| | | LEU A:409 | Alkyl | 4.19 | |
| | | VAL A:296 | Alkyl | 4.06 | |
| | | LEU A:295 | Alkyl | 5.30 | |
| | | VAL A:445 | Alkyl | 5.37 | |
| | | HIS A:389 | Pi-Alkyl | 4.65 | |
| | | PHE A:405 | Pi-Alkyl | 5.30 | |

**Table no-2 Paracetamol with COX-2 (4PH9 protein) binding energy and related aminoacids:-**

| Ligand Name | PDB Code | Amino acid residue | Nature of Interaction | Binding Distance(Å) | Binding Energy (kcal/mol) |
|---|---|---|---|---|---|
| Paracetamol | 4PH9 | ALA A:200 | Conventional Hydrogen Bond | 2.82 | -6.3 |
| | | TYR A:386 | Conventional Hydrogen Bond | 1.92 | |
| | | HIS A:387 | Pi-Sigma | 3.78 | |
| | | HIS A:389 | Pi –Pi T Shaped | 5.17 | |
| | | ALA A:203 | Pi-Alkyl | 5.12 | |

**Table no-3 Silymarin with COX-2 (4PH9 protein) binding energy and related aminoacids:-**

| Ligand Name | PDB Code | Amino acid residue | Nature of Interaction | Binding Distance (Å) | Binding Energy(kcal/mol) |
|---|---|---|---|---|---|
| Silymarin | 4PH9 | ASN A:145 | Conventional Hydrogen Bond | 2.15 | -10.2 |
| | | SER A:147 | Conventional Hydrogen Bond | 3.63 | |
| | | ARG A:377 | Conventional Hydrogen Bond | 3.02 | |
| | | GLN A:375 | Conventional Hydrogen Bond | 3.02 | |
| | | LEU A:146 | Carbon Hydrogen Bond | 4.02 | |
| | | ARG B:377 | Unfavorable Donor-Donor | 2.37 | |
| | | SER A:144 | Pi -Donor Hydrogen Bond | 3.71 | |
| | | LEU B:225 | Alkayl | 4.14 | |

**Table no-4 Nonadecanoicacid with P-450 (3GPH protein) binding energy and related aminoacids:-**

| Ligand Name | PDB Code | Amino acid residue | Nature of Interaction | Binding Distance (Å) | Binding Energy (kcal/mol) |
|---|---|---|---|---|---|
| Nonadecanoic acid | 3GPH | ASN A:206 | Conventional Hydrogen Bond | 3.11 | -8.1 |
| | | VAL A:239 | Carbon Hydrogen Bond | 3.13 | |
| | | ILE A:361 | Carbon Hydrogen Bond | 3.44 | |
| | | PHE A:207 | Pi- Sigma | 3.87 | |
| | | VAL A:365 | Alkyl | 3.52 | |
| | | ILE A:115 | Alkyl | 4.06 | |
| | | LEU A:368 | Alkyl | 4.79 | |
| | | ALA A:299 | Alkyl | 4.21 | |
| | | PHE A:478 | Pi-Alkyl | 5.19<br>4.68 | |
| | | PHE A:298 | Pi-Alkyl | 4.86<br>4.06 | |
| | | PHE A:478 | Pi-Alkyl | 4.68 | |

**Table no-5 Paracetamol with P-450 (3LC4 protein) binding energy and related aminoacids**

| Ligand Name | PDB Code | Amino acid residue | Nature of Interaction | Binding Distance(Å) | Binding Energy (kcal/mol) |
|---|---|---|---|---|---|
| Paracetamol | 3LC4 | ASN A:206 | Conventional Hydrogen Bond | 3.29 | -7 |
| | | PHE A:207 | Pi-Donor Hydrogen Bond | 2.91 | |
| | | PHE A:207 | Pi-Sigma | 3.45 | |
| | | PHE A:298 | Pi-Pi Stacked | 3.90 | |

**Table no- 6 Silymarin with P-450 (3T3Z protein) binding energy and related aminoacids:-**

| Ligand Name | PDB Code | Amino acid residue | Nature of Interaction | Binding Distance (Å) | Binding Energy (kcal/mol) |
|---|---|---|---|---|---|
| Silymarin | 3T3Z | THR D:307 | Conventional Hydrogen Bond | 2.07 | -10.8 |
| | | CYS D:437 | Pi-Donor Hydrogen Bond | 4.09 | |
| | | VAL D:364 | Alkyl | 3.78 | |
| | | LEU D:363 | Pi-Alkyl | 4.34 | |
| | | ALA D:299 | Pi-Alkyl | 5.28 | |
| | | LEU D:368 | Pi-Alkyl | 4.72 | |
| | | ILE D:115 | Pi-Alkyl | 4.72 | |
| | | PHE D:207 | Pi-Alkyl | 4.84 | |
| | | PRO D:429 | Pi-Alkyl | 4.79 | |

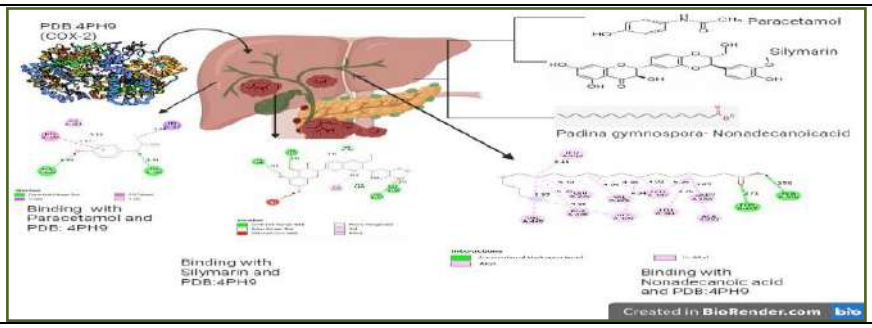



**Figure :1 Graphical abstract the hepatoprotective of *Padina gymnospora***

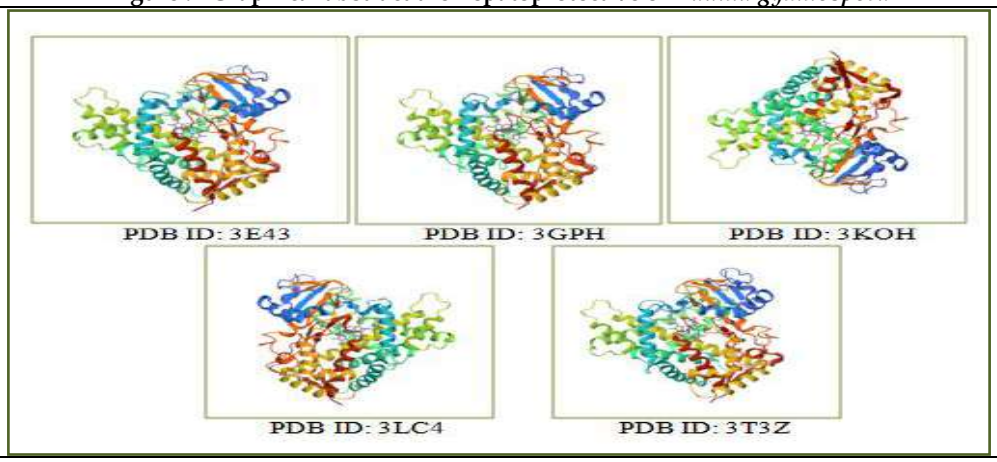



**Figure 2 Types of proteins CYP2E1**

**(i)** **(ii)**

**Figure:2(A) (i) Map:- *Padina gymnospora* founds Gulf of Mannar,Tuticorin located (8.76°42″ N; 78.13° 48″ E) at Rameswaram in Tamil Nadu in India; (ii) Herbarium sheet-Fan shaped thallus.**

**Figure : 3 Online CB Dock-2 process is showing**

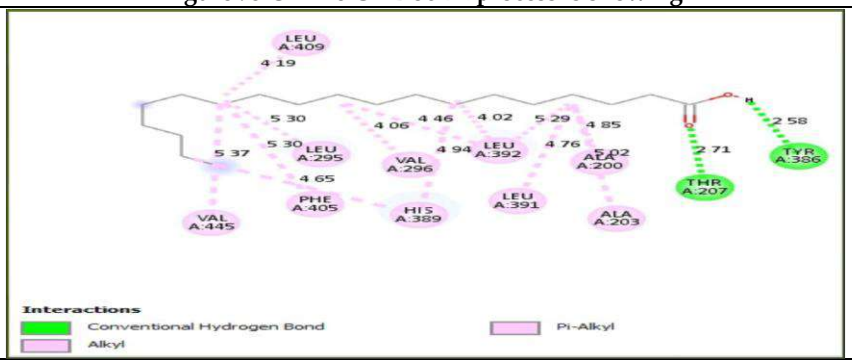



**Figure 4. 2D structure of Nonadecanoic acid with 4PH9 protein**

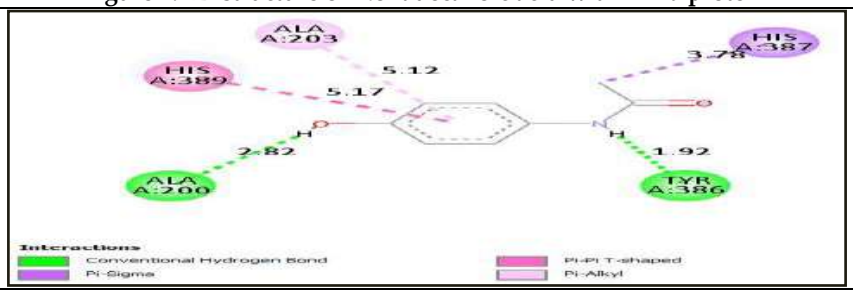



**Figure 5 . 2D structure of Paracetamol with 4PH9 protein**

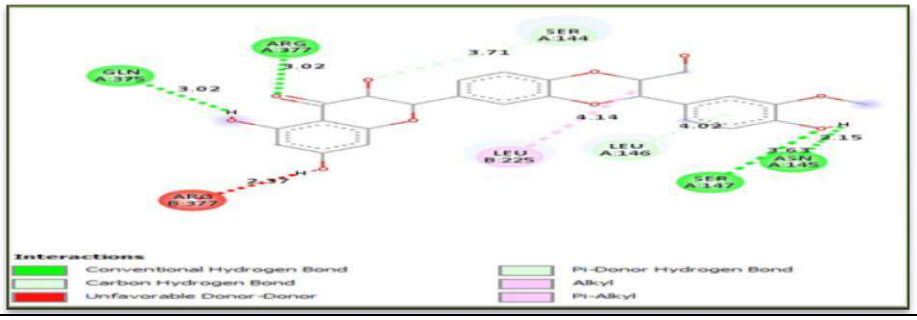



**Figure 6. 2D structure of Silymarin with 4PH9 protein**

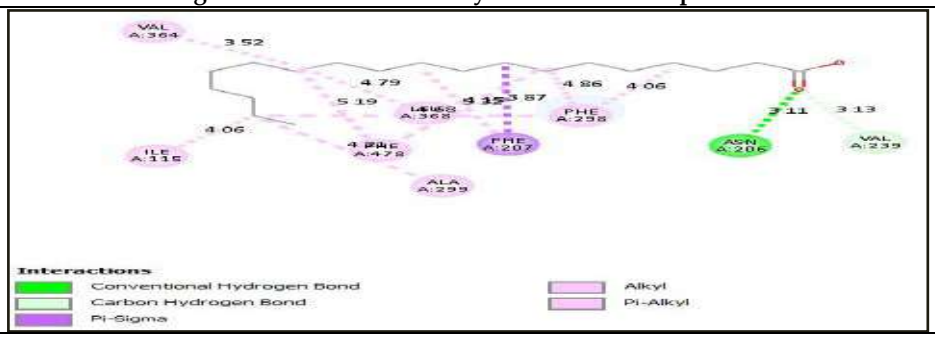



**Figure 7. 2D structure of Nonadecanoic acid with 3GPH protein**

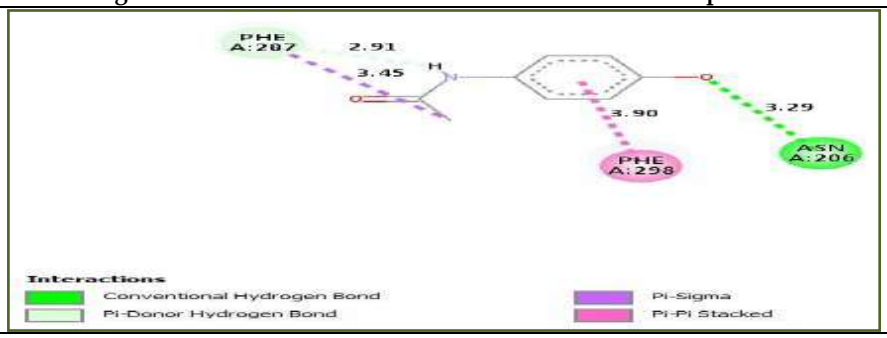



**Figure 8. 2D structure of Paracetamol with 3LC4 protein**

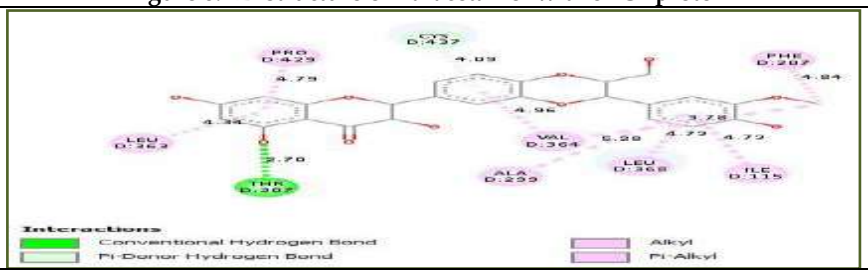
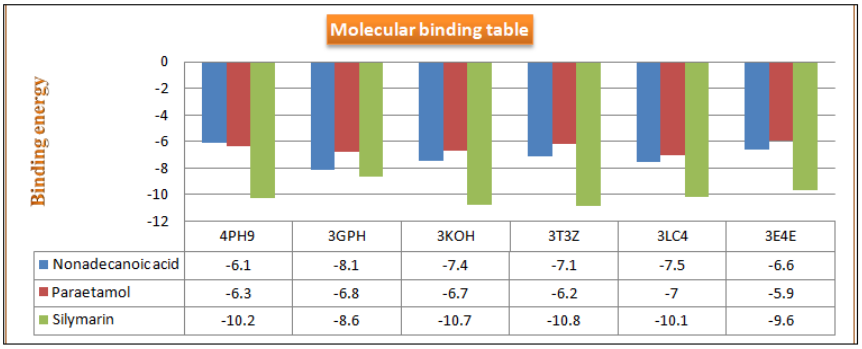



**Figure 9. 2D structure of Silymarin with 3T3Z protein**




| | 4PH9 | 3GPH | 3KOH | 3T3Z | 3LC4 | 3E4E |
|---|---|---|---|---|---|---|
| Nonadecanoic acid | -6.1 | -8.1 | -7.4 | -7.1 | -7.5 | -6.6 |
| Paraetamol | -6.3 | -6.8 | -6.7 | -6.2 | -7 | -5.9 |
| Silymarin | -10.2 | -8.6 | -10.7 | -10.8 | -10.1 | -9.6 |

**Figure 10. Graphical represented of molecular binding (protein ligand bond)**

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**RESEARCH ARTICLE**

# Drug Solubility: Importance and Enhancement Strategies

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**ABSTRACT**

Solubility, the process of dissolving a solute in a solvent to form a homogeneous mixture, plays a critical role in achieving the desired drug concentration in systemic circulation to elicit the anticipated pharmacological effect. Poor aqueous solubility poses a significant challenge in the formulation of both new chemical entities (NCEs) and generic drugs. Over 40% of NCEs developed by the pharmaceutical industry are practically insoluble in water. The Biopharmaceutical Classification System (BCS) categorizes compounds based on their solubility and permeability. This classification is widely adopted by regulatory authorities and health organizations to utilize dissolution testing as evidence of bioequivalence for substances that are highly soluble and highly permeable. For formulation scientists, improving solubility is a key hurdle, as drugs must exist in a dissolved state at the absorption site for effective uptake. Various approaches, including physical and chemical modifications of the drug and techniques like particle suspension, are employed to enhance the solubility of poorly soluble drugs.

**Keywords:** solubility, chemical, permeable, Classification System.

## INTRODUCTION

Solubility refers to the ability of a solid, liquid, or gaseous substance, known as the solute, to dissolve in a solid, liquid, or gaseous solvent, resulting in a homogeneous solution. The solubility of a substance is primarily influenced by the choice of solvent, as well as factors such as temperature and pressure. It is typically measured by the saturation point, where adding more solute does not increase the concentration of the solution.[1].

In scientific terms, solubility represents the maximum amount of a substance that can dissolve in a specified volume of solvent. It can be described both qualitatively and quantitatively. Qualitatively, solubility is the natural interaction between two or more substances to form a uniform mixture. Quantitatively, it refers to the concentration of the solute in a solvent at a particular temperature, resulting in a homogeneous solution. In pharmaceutical contexts, solubility may be expressed in various units, including percentages, parts per quantity, molarity, molality, mole fraction, or volume fraction. [2]. Solubility plays a crucial role in pharmaceuticals, especially for drugs with poor water solubility, such as those in Biopharmaceutical Classification System (BCS) classes II and IV, which often encounter challenges related to dissolution. [3]. The phenomenon of solubility occurs at dynamic equilibrium, where two opposing processes—dissolution and phase separation (e.g., precipitation)—take place at equal rates. This balance creates a state known as solubility equilibrium. Under specific conditions, the equilibrium solubility can be surpassed, leading to a metastable supersaturated solution.

IUPAC defines solubility as the analytical composition of a saturated solution expressed as a proportion of a designated solute in a designated solvent. Solubility may be stated in units of concentration, molality, mole fraction, mole ratio, and other units [4]. The concept of solubility is applied in various contexts, leading to its expression through multiple measures. Solubility is commonly quantified as a concentration, such as grams of solute per kilogram of solvent, grams per deciliter (100 mL) of solvent, molarity, molality, or mole fraction. The solubility of a substance represents the maximum amount of solute that can dissolve in a given quantity of solvent under specified conditions. This approach is straightforward but can be influenced by other components in the solvent, such as the common ion effect. [5]. For ionic compounds with limited solubility, solubility is often described using solubility constants. This equilibrium-based measure represents the balance between dissolved ions and undissolved salt. Like other equilibrium constants, the solubility constant is temperature-dependent but is generally unaffected by the presence of other species in the solvent. The Biopharmaceutics Classification System (BCS), developed by the U.S. Food and Drug Administration, predicts intestinal drug absorption based on solubility and intestinal permeability. Solubility is determined by the highest-dose strength of an immediate-release product. A drug is classified as highly soluble if its highest dose strength can dissolve in 250 mL or less of aqueous media across a pH range of 1 to 7.5. This volume is based on bioequivalence study protocols where drugs are administered with a standard glass of water to fasting volunteers. [6]

The classification of intestinal permeability is based on comparisons with intravenous administration. These factors are critical since 85% of the most widely sold drugs in the U.S. and Europe are orally administered.
Drugs are categorized into four classes under the BCS:

- **Class I**: High solubility and high permeability
- **Class II**: Low solubility and high permeability
- **Class III**: High solubility and low permeability
- **Class IV**: Low solubility and low permeability

**Significance of Solubility**

Oral drug delivery is the most widely used and convenient route for medication administration due to its ease of use, high patient compliance, cost-effectiveness, minimal sterility requirements, and flexibility in designing dosage forms. Consequently, many generic pharmaceutical companies focus on developing bioequivalent oral drug products. [7]. However, a key challenge in formulating oral dosage forms is achieving optimal bioavailability. Oral bioavailability is influenced by factors such as aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, presystemic metabolism, and susceptibility to efflux mechanisms. Among these, poor solubility and low permeability are the most common causes of reduced bioavailability. [8]. Solubility is not only critical for oral dosage forms but also plays a significant role in other formulations, including parenteral preparations. It is a fundamental parameter for achieving the desired drug concentration in systemic circulation, which is essential for eliciting the intended pharmacological effect. Drugs with poor water solubility often require higher doses to achieve therapeutic plasma concentrations after oral administration. [9]

The development of formulations for poorly water-soluble drugs remains a significant challenge in both new chemical entity (NCE) research and generic drug development. For a drug to be absorbed, it must exist in an aqueous solution at the site of absorption. Since water is the preferred solvent for liquid pharmaceutical formulations, poor aqueous solubility becomes a major hurdle, especially as many drugs are weak acids or weak bases with limited solubility in water. [10]. Over 40% of NCEs developed by the pharmaceutical industry are practically insoluble in water. This low solubility results in slow absorption, inconsistent bioavailability, and sometimes gastrointestinal mucosal toxicity. For orally administered drugs, solubility is often the primary rate-limiting factor in achieving the desired systemic drug concentration for therapeutic action. Addressing solubility issues remains a critical focus for formulation scientists.

Enhancing drug solubility and consequently improving oral bioavailability remains one of the most significant challenges in drug development, particularly for oral delivery systems. Various strategies have been explored and documented in the literature to address the solubility issues of poorly water-soluble drugs. The choice of technique depends on factors such as the physicochemical properties of the drug, the nature of the excipients, and the intended dosage form. Poor solubility and slow dissolution rates in the aqueous gastrointestinal environment often result in inadequate bioavailability for such drugs. This issue is particularly relevant for Biopharmaceutics Classification System (BCS) Class II drugs, which are characterized by low solubility but high permeability. For these drugs, the rate-limiting factor is not absorption but rather drug release from the dosage form and solubility in gastric fluids. Therefore, enhancing solubility and dissolution rates can significantly improve the bioavailability of BCS Class II compounds. [10]. Low-solubility drugs pose several challenges, including poor absorption and bioavailability, limited solubility for intravenous administration, prolonged development timelines, increased costs, and a higher dosing burden on patients, often requiring frequent administration of large doses. Addressing these solubility challenges is crucial for the efficient development of effective and patient-friendly drug formulations.

**Methods to Enhance Solubility**

Solubility improvement techniques can be categorized in to physical modification, chemical modifications of the drug substance, and other techniques.

**Physical Modifications**

Particle size reduction like micronization and nanosuspension, modification of the crystal habit like polymorphs, amorphous form and cocrystallization, drug dispersion in carriers like eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques.

**Chemical Modifications**

Change of ph, use of buffer, derivatization, complexation, and salt formation.

**Miscellaneous Methods**

Supercritical fluid process, use of adjuvant like surfactant, solubilizers, cosolvency, hydrotrophy, and novel excipients.

**Particle Size Reduction**

The solubility of a drug is closely linked to its particle size. Reducing particle size increases the surface area, which enhances the drug's dissolution properties. For poorly soluble drugs, particle size reduction often correlates with improved bioavailability. Techniques such as milling, using tools like colloid mills and jet mills, are commonly employed to reduce particle size. However, while these methods improve dissolution rates, they do not alter the drug's saturation solubility, making them unsuitable for drugs with a high dose number. Particle size reduction can also be achieved through advanced techniques like nanosuspension and micronization. [11]

**Micronization**

Micronization involves reducing the particle size to enhance the dissolution rate of drugs by increasing the surface area of the particles. However, it does not increase the equilibrium solubility. By decreasing particle size, the rate of dissolution improves, leading to better absorption and bioavailability. This technique has been applied to drugs such as progesterone, fenofibrate, griseofulvin, spironolactone, and diosmin, resulting in enhanced bioavailability, improved digestive absorption, and greater clinical efficacy. [12]

**Modification of Crystal Habit**

Crystal habit modification involves altering the solid-state forms of drugs, including polymorphs and pseudopolymorphs:

**Polymorphs**: These are different crystalline forms of the same drug, characterized by distinct arrangements in the crystal lattice. While chemically identical, polymorphs exhibit different physicochemical properties, such as melting point, density, solubility, stability, and texture.

**Pseudopolymorphs**: These refer to solvates or hydrates, where solvents are incorporated into the crystal lattice.
Amorphous forms of drugs, which lack a well-defined crystalline structure, often exhibit better solubility and dissolution rates compared to crystalline forms. This is due to their higher surface area and greater associated energy. The solubility hierarchy for solid drug forms is typically as follows:

**Amorphous > Metastable Polymorphs > Stable Polymorphs**

**Solid Dispersion**

The concept of solid dispersions was first introduced by Sekiguchi and Obi in the 1960s when they explored the dissolution and performance of eutectic mixtures of sulfonamide drugs with water-soluble carriers. Solid dispersion is a versatile pharmaceutical approach to enhance the dissolution rate, absorption, and therapeutic efficacy of poorly water-soluble drugs in dosage forms.Solid dispersions consist of at least two components: a hydrophilic matrix and a hydrophobic drug. Common hydrophilic carriers used in solid dispersions include polyvinylpyrrolidone (PVP), polyethylene glycols (PEGs), and Plasdone-S630. Surfactants such as Tween-80, docusate sodium, Myrj-52, Pluronic-F68, and sodium lauryl sulfate (SLS) are also incorporated into these formulations to enhance performance. [13]. The solubility of drugs like celecoxib, halofantrine, and ritonavir has been improved using solid dispersion techniques. For instance, celecoxib with PVP and ritonavir with Gelucire have demonstrated increased aqueous solubility. Key techniques for preparing solid dispersions include:

- **Fusion Method**: In this approach, the hydrophilic carrier is heated above its melting point, and the drug is incorporated into the molten carrier with continuous stirring. The mixture is then cooled to allow the drug to be evenly dispersed throughout the matrix.
- **Solvent Evaporation Method**: The drug and carrier are dissolved in an appropriate organic solvent. The solvent is then evaporated under vacuum and elevated temperatures to yield a solid residue containing the dispersed drug.

**Nanosuspension[14]**

Nanosuspension technology has emerged as a promising strategy for the effective delivery of hydrophobic drugs, especially those insoluble in both water and oils. A nanosuspension is a biphasic system composed of nanometer-sized drug particles stabilized by surfactants, and it is suitable for oral, topical, parenteral, and pulmonary administration. The solid particles in a nanosuspension typically have a particle size of less than one micron, with an average size ranging from 200 to 600 nm. Various techniques for the preparation of nanosuspensions include:

**Precipitation Technique**: Involves reducing the drug particle size by precipitation in a suitable solvent system.

**Media Milling**: Utilizes high-energy milling to achieve nano-sized particles.
**High-Pressure Homogenization in Water**: Drug particles are reduced to the nanoscale by applying high pressure in an aqueous medium.
**High-Pressure Homogenization in Nonaqueous Media**: The same process is applied using nonaqueous media for drugs with special stability requirements.
**Combination of Precipitation and High-Pressure Homogenization**: Combines the benefits of both techniques to achieve optimal particle size and stability.
Nanosuspension technology addresses the challenges of poor solubility and enhances the bioavailability of hydrophobic drugs, offering versatility across different routes of administration.

**Supercritical Fluid (SCF) Process[15]**
The supercritical fluid (SCF) process is an innovative nanosizing and solubilization technique that has gained significant attention in recent years for particle size reduction. A supercritical fluid is defined as a substance at a temperature and pressure above its critical point, allowing it to exhibit the combined properties of gases and liquids. At near-critical temperatures, SCFs become highly compressible, enabling slight changes in pressure to significantly alter their density and mass transport characteristics, which are crucial in determining their solvent capabilities.
In SCF processes, drug particles are dissolved in a supercritical fluid, most commonly carbon dioxide, and subsequently recrystallized into much smaller sizes. This technique offers precision and flexibility, allowing for the production of drug particles with sizes in the submicron range. Current SCF technologies are capable of producing nanoparticulate suspensions with particle sizes ranging from 5 to 2,000 nm.
Several pharmaceutical companies, such as Nektar Therapeutics and Lavipharm, specialize in using SCF technologies for particle size reduction and solubility enhancement. Various SCF techniques have been developed to optimize specific aspects of the process, including:

- Precipitation with Compressed Antisolvent Process (PCA)
- Solution-Enhanced Dispersion by SCF (SEDS)
- Supercritical Antisolvent Process (SAS)
- Rapid Expansion of Supercritical Solutions (RESS)
- Gas Antisolvent Recrystallization (GAS)
- Aerosol Supercritical Extraction System (ASES)

These methods enhance solubility and dissolution rates by producing uniform particles with improved bioavailability.

**Cryogenic Techniques[16,17]**
Cryogenic techniques are advanced methods aimed at improving the dissolution rate of drugs by creating nanostructured amorphous particles with high porosity under extremely low-temperature conditions. These techniques utilize various cryogenic injection devices, including capillary, rotary, pneumatic, and ultrasonic nozzles. Depending on the system design, the nozzles may be positioned above or below the liquid level, and the cryogenic liquids employed can include hydrofluoroalkanes, nitrogen ($N_2$), argon (Ar), oxygen ($O_2$), or organic solvents.
Following cryogenic processing, the resulting dry powders are typically obtained through drying processes such as spray freeze drying, atmospheric freeze drying, vacuum freeze drying, or lyophilization.
Key cryogenic techniques include:

1. **Spray Freezing onto Cryogenic Fluids**: A process where the drug is atomized into cryogenic fluids to create nanostructured particles.
2. **Spray Freezing into Cryogenic Liquids (SFL)**: The drug solution is sprayed directly into a cryogenic liquid to rapidly solidify the particles.

3. **Spray Freezing into Vapor over Liquid (SFV/L)**: Drug particles are formed by spraying into the vapor phase above a cryogenic liquid.
4. **Ultra-Rapid Freezing (URF)**: This technique uses extremely rapid cooling rates to produce highly porous, nanostructured drug particles.

Cryogenic methods offer significant advantages for enhancing solubility and dissolution by reducing particle size and increasing surface area, making them an effective approach for poorly water-soluble drugs.

**Inclusion Complex Formation-Based Techniques[18]**

Inclusion complex formation is a widely utilized method for enhancing the aqueous solubility, dissolution rate, and bioavailability of poorly water-soluble drugs. This technique involves the encapsulation of a nonpolar molecule or its nonpolar region (the guest) into the cavity of another molecule or group of molecules (the host). Cyclodextrins are the most commonly used host molecules in this process. Cyclodextrins (CDs) are cyclic oligomers produced through the enzymatic degradation of starch by cyclodextrin-glycosyltransferase (CGT). The three naturally occurring types of cyclodextrins are $\alpha$-Cyclodextrin, $\beta$-Cyclodextrin, and $\gamma$-Cyclodextrin. While the hydrophilic exterior of cyclodextrins makes them soluble in water, their hydrophobic cavities provide an ideal microenvironment for accommodating nonpolar molecules. Several techniques have been developed to prepare inclusion complexes of poorly water-soluble drugs with cyclodextrins, including:

1. **Kneading Method**: Mixing the drug and cyclodextrin in a small amount of solvent to form a paste, followed by drying.
2. **Lyophilization/Freeze-Drying Technique**: Dissolving both the drug and cyclodextrin in a suitable solvent, freezing the solution, and removing the solvent by sublimation.
3. **Microwave Irradiation Method**: Using microwave energy to facilitate the formation of inclusion complexes by heating and mixing the drug and cyclodextrin in a solvent.

**Micellar Solubilization[19]**

Micellar solubilization is one of the oldest and most fundamental techniques for improving the solubility and dissolution rates of poorly soluble drugs. This method involves the use of surfactants to reduce surface tension, which enhances the dissolution of lipophilic drugs in aqueous media. Surfactants are also employed to stabilize suspensions of drugs and improve the wetting of solid drug particles. When the concentration of surfactants exceeds their critical micelle concentration (CMC), which typically ranges from 0.05% to 0.10%, micelles form. These micelles can encapsulate poorly soluble drugs within their hydrophobic cores, significantly enhancing solubility. In addition, surfactants aid in the disintegration of solid drug particles into finer particles, further improving dissolution rates.

Common nonionic surfactants used in micellar solubilization include:

- Polysorbates
- Polyoxyethylated castor oil
- Polyoxyethylated glycerides
- Lauroyl macroglycerides
- Mono- and di-fatty acid esters of low molecular weight polyethylene glycols

Surfactants are also frequently used to stabilize microemulsions and suspensions, which serve as carriers for solubilized drugs. Examples of poorly soluble drugs that have benefited from micellar solubilization include antidiabetic medications such as gliclazide, glyburide, glimepiride, glipizide, repaglinide, pioglitazone, and rosiglitazone. This technique has proven effective in improving the bioavailability and therapeutic efficacy of such compounds.

**Hydrotropy[20]**

Hydrotropy is a solubilization technique in which the addition of a large quantity of a second solute, known as a hydrotropic agent, enhances the aqueous solubility of a poorly soluble substance. Hydrotropic agents are typically ionic organic salts, often composed of alkali metal salts of various organic acids. These agents either increase solubility in a solvent ("salting in") or decrease it ("salting out"). Salts with large anions or cations that are highly soluble in water can lead to the "salting in" of non-electrolytes, a phenomenon referred to as "hydrotropism." Hydrotropy thus describes the enhancement of solubility in water through the presence of a significant amount of additives. The mechanism underlying hydrotropy involves weak interactions, such as complexation, between the hydrotropic agents (e.g., sodium benzoate, sodium acetate, sodium alginate, urea) and the poorly soluble drugs. Hydrotropes are known to self-assemble in solution, contributing to their solubilization effect.

Classifying hydrotropes based on molecular structure is challenging because a wide range of compounds exhibit hydrotropic behavior. Common examples include:

- **Alcohols**: Ethanol, resorcinol, pyrogallol, catechol
- **Aromatic alcohols**: $\alpha$- and $\beta$-naphthols, salicylates
- **Alkaloids**: Caffeine, nicotine
- **Ionic surfactants**: Diacids, sodium dodecyl sulfate (SDS), dodecylated oxidibenzene

Among these, aromatic hydrotropes with anionic head groups are the most extensively studied. Their efficacy is often attributed to the interactive $\pi$ (pi) orbitals available in their structure. Conversely, hydrotropes with cationic hydrophilic groups are rare, but examples include salts of aromatic amines such as procaine hydrochloride. In addition to improving solubility, hydrotropes influence surfactant behavior by enhancing micelle formation, altering the phase behavior of multicomponent systems, and affecting nanodispersions, conductance percolation, surfactant clouding, and polymer interactions. These diverse effects make hydrotropy a valuable tool in the development of solubility-enhancing formulations.

**Crystal Engineering[21,22]**

The surface area of a drug available for dissolution is influenced by its particle size and its ability to be wetted by luminal fluids. The particle size, which directly affects the dissolution rate of the drug, is determined by crystallization conditions or methods such as impact milling and fluid energy milling. Comminution techniques can produce particles that are highly heterogeneous, charged, and cohesive, potentially causing issues in downstream processing and product performance. To address these challenges, crystal engineering techniques have been developed to enable the controlled crystallization of drugs, resulting in high-purity powders with well-defined particle size distribution, crystal habit, crystal form (either crystalline or amorphous), surface characteristics, and surface energy. By manipulating crystallization conditions—such as altering solvents, changing stirring rates, or adding other components to the drug solution—it is possible to prepare crystals with different packing arrangements. These different forms are called polymorphs.

Crystal engineering provides various routes to improve solubility and dissolution rates through a deep understanding of crystallization processes and the molecular properties of active pharmaceutical ingredients. The process often involves dissolving the drug in a solvent and controlling its precipitation to produce nanoparticles, typically through the addition of an antisolvent (commonly water) Pharmaceutical cocrystals represent a significant advancement in addressing the solubility issues of poorly soluble drugs. These cocrystals consist of two or more distinct molecules arranged to create a new crystal form with properties superior to those of the separate entities. Pharmaceutical cocrystals are formed between a molecular or ionic drug and a cocrystal former that remains solid under ambient conditions. Cocrystals are typically prepared through slow evaporation from a drug solution containing stoichiometric amounts of the components (cocrystal formers), although other methods such as sublimation, melt growth, or grinding in a ball mill are also viable .

Traditional crystallization techniques, such as sublimation, crystallization from solutions, evaporation, thermal treatment, desolvation, grinding, and milling, continue to be employed alongside other methods like salt formation, altering the dielectric constant of solvents, chemical modification, the use of hydrates or solvates, soluble prodrugs, and application of ultrasonic waves or spherical crystallization, to enhance the solubility of poorly water-soluble drugs.

## CONCLUSION

In this review paper, we emphasize that the solubility of a molecule is fundamental and plays a crucial role in pharmaceutical formulation and development. The rate of oral absorption of weakly water-soluble drugs is primarily influenced by drug dissolution, making solubility an essential factor in the formulation and development of various dosage forms. The techniques discussed can be used individually or in combination to enhance the solubility of drugs. The proper selection of a solubility enhancement method is vital to achieving key formulation goals such as improved oral bioavailability, reduced dosing frequency, enhanced patient compliance, and cost-effective production. The choice of solubility enhancement method depends on several factors, including the drug's characteristics—such as solubility, chemical nature, melting point, absorption site, and pharmacokinetic behavior. Additionally, the method must align with the dosage form requirements (e.g., tablet or capsule, immediate or modified release), and meet regulatory standards regarding excipient use, maximum daily dose, approved excipients, and analytical accuracy.

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**RESEARCH ARTICLE**

# High Performance Liquid Chromatography for the Development and Validation of Meropenem and Vaborbactam

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## ABSTRACT

Meropenem and vaborbactam were analyzed and quantified using a reverse-phase high-performance liquid chromatography (RP-HPLC) technology that was developed and verified to be quick, sensitive, and reliable. A photodiode array (PDA) detector Waters HPLC system was used for the analysis. Using an Inertsil ODS C18 column (250 × 4.6 mm, 5 μm) at a flow rate of 1.0 mL/min, chromatography was performed. The detection was carried out at a wavelength of 252 nm, and the mobile phase was a degassed combination of methanol and buffer (80:20, v/v). Vaborbactam had a retention duration of 6.691 minutes, whilst Meropenem showed a retention time of 4.713 minutes. ICH guidelines were followed in the rigorous testing of each of these parameters: System Suitability, Specificity, LOD & LOQ, Linearity, Recovery, Precision, Ruggedness, and Robustness

**Keywords:** Meropenem, Vaborbactam, RP-HPLC, Method Development, Validation.

## INTRODUCTION

Patient safety is seriously at stake due to the alarming rate at which the overall danger of antibiotic resistance is growing. The World Health Organization (WHO) has named carbapenem-resistant Enterobacteriaceae (CRE) as one of the three most important infections that require the development of new antibiotic treatments, and the Centers for Disease Control and Prevention has designated CRE as a "urgent" danger [1–3]. In the past, patients with invasive CRE infections have had up to 70% mortality rates. This emphasizes how urgently new therapeutic alternatives must be found and developed, especially for gram-negative bacteria.The FDA recently approved Meropenem-Vaborbactam (Vabomere™), a carbapenem antibiotic and a first-in-class β-lactamase inhibitor based on boronic acid, to treat complex urinary tract infections, including pyelonephritis, in response to this urgent need [4]. The molecular name for meropenem is (4R,5S,6S).-3-(((3S,5S))6-((R)-1-hydroxyethyl)-5-(dimethylcarbamoyl)pyrrolidin-3-yl)thio)4-methylSeven-oxo1-azabicyclo[3.2.0]A sulfur-to-carbon substitution at position 1 and an unsaturated link between C2 and C3 in the penicillin core structure (Fig. 1a) are two important structural characteristics that hept-2-ene-2-carboxylic acid, a carbapenem antibiotic, shares with other medications in this family [5]. The action of Meropenem against gram-negative bacteria is increased by the dimethyl-carbamoylpyrrolidinethio side chain at position C2. Meropenem, like other carbapenems, inhibits the formation of cell walls by attaching itself to penicillin-binding proteins (PBPs). Meropenem may offer a stronger antibacterial backbone than cephalosporins when used with carbapenemase inhibitors.

The molecular name for vaborbactam is (3R,6S)-2-Hydroxy-3-[2-(thiophen-2-yl)acetamido].A new non-β-lactam cyclic boronic acid inhibitor of β-lactamases, -1,2-oxaborinan-6-yl} acetic acid is based on a cyclic boronic acid pharmacophore (Figure 2) [5]. When it comes to Klebsiella pneumoniae carbapenemase (KPC), VAB is especially effective. A conformation that preferentially increases its action against β-lactamases is designed into its cyclic boronate ester structure. By adding a 2-thienyl acetyl group, Vaborbactum's efficiency as a β-lactamase inhibitor is increased and its potency is further enhanced [6]. Vaborbactam mainly targets class A and C β-lactamase enzymes, such as the Serratia marcescens enzyme and those that cause imipenem resistance [7]. Figures 1 and 2 depict the structures of vaborbactam and meropenem.

## MATERIALS AND METHODS

**Materials and Reagents**

On the day of preparation, meropenem and vaborbactam were obtained, the solutions were made, well-protected from the effects of light, and analyzed. The glassware used in the procedure was carefully cleaned using a solution of sulfuric and chromic acids, rinsed with double-distilled water, and then dried in a hot air oven. Analytical-reagent (AR) grade reagents were utilized. Acetonitrile, Methanol HPLC Grade, and Buffer (KH2PO4) HPLC Grade were purchased from Merck, India.

**Instrumentation**

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used for the chromatographic analysis on a Waters Model 2690/5 Series Compact System that had an Inertsil-C18 ODS column installed. A Rheodyne injector with a 20 μL loop made up the sample injection apparatus. Empower 2.0 software was used to record and evaluate the data. An electronic scale (SARTORIUS) was utilized for the purpose of weighing, and the medication and sample solutions were sonicated using a sonicator (FAST CLEAN).

**Chromatographic Conditions**

Using a mobile phase composition of 80:20 V/V with acetonitrile and methanol at a flow rate of 1.0 ml/min, the analyte separation was performed on the Inertsil-ODS C18 (250 x 4.6 mm, 5) column. The eluents were detected at 252 nm with an injection volume of 20 μL. A room temperature of 30°C was used for the chromatographic analysis.

**Preparation of Buffer($KH_2PO_4$ 0.1 M)**

3.8954 grams of di-sodium hydrogen phosphate and 3.4023 grams of potassium dihydrogen phosphate should dissolve completely in a beaker filled with 1000 milliliters of pure water. After that, the pH is adjusted using orthophosphoric acid and passed through a 0.45µm membrane filter.

**Solution for Stocks Preparation**

Reference solution: In two 100.0 mL volumetric flasks, 20.0 mg of Meropenem and 25.0 mg of Vaborbactam were dissolved in a mobile process, and the mixture was then sonicated for 20 minutes each. Put 10.0 mL of each of the previously indicated solutions into a 50.0 mL volumetric flask, mix with the mobile phase for 10 minutes, and then sonicate.

**Standard Working Solution Preparation**

Together with Meropenem and Vaborbactam, stock solutions ranging from 20 ppm to 80 ppm for both medications were created, sonicated, and filtered through a 0.45 membrane.

**Method Validation**

Following the ICH recommendations, the procedure was validated under optimal chromatographic conditions [17–21]. Stability, specificity, recovery, linearity, accuracy, precision, robustness, robustness LOD, and LOQ were all validated for the parameter system suitability test.

## RESULTS

**Optimization of chromatographic condition**

The UV spectra of Meropenem and Vaborbactam showed a significant absorption at 252 nm, which was chosen as the analytical wavelength. Numerous combinations were tested using varying acetonitrile and methanol compositions as the mobile phase. To resolve Meropenem and Vaborbactam and to achieve appropriate retention durations and peak symmetry, preliminary experiments were conducted using various mixtures of acetonitrile and methanol. In order to get acceptable system compatibility characteristics, a mobile phase comprising 80:20 V/V with methanol and buffer, Inertsil-ODS C18 (250 x 4.6 mm, 5) column was finally chosen. We experimented with flow rates ranging from 0.5 to 1.2 ml/min. A flow rate of 1.0 ml/min was proved to be better as with both drugs have retention time less than 10 min. The optimized chromatogram Figure. 3 The retention time of Meropenem and Vaborbactam were found to be 4.713 and 6.691 min respectively.

**Method Validation**

**System Suitability Test**

System suitability parameters for the new method were calculated from the optimized chromatographic settings and compared with suggested limits. Table 1 illustrated the method's system suitability parameters. The findings showed that the approach was appropriate for the analysis and that all of the system suitability parameters fell within the suggested ranges.

**Linearity**

A range of solutions with concentration levels ranging from 20 ppm to 80 ppm of the target concentration are created using the working standards for vaborbactam and meropenem.The device's linear fit was illustrated graphically. Table 2 presents the results (a-b). In Figure 4(a-b), the linearity curve was displayed.

**Repeatability(Repetition)**

a. System precision (system precision): Five injections of a standard solution were made using the test procedure.
b. Technique precision (precision process): Each solution was injected separately, and six sample preparations were generated independently using a single test procedure.

The results of the tests show that the research technique is valid. The accuracy of the system and technique may be found in Tables 3-5.

**Intermediate accuracy (variable analyst):**

Two analysts conducted a test technique analysis. Both the individual percentage assays and the percentage RSD of the assay pass the intermediate accuracy test and fall within the allowed range. Table 5(i-ii).

**Recovery**

An accuracy investigation was carried out. Using the test method in triplicate, with an equivalent amount of Meropenem and Vaborbactam in each volumetric flask for each spike level, the concentration of Meropenem and Vaborbactam equal to 50%, 100%, and 150% of the labeled quantity was ascertained. Meropenemand Vaborbactam's average recovery rate was determined.

.

$$\% \, Recovery = \frac{Amount\ found}{Amount\ added} \, X\ 100$$

The findings of the recovery show that the test procedure has an appropriate degree of precision. Refer Table 6.

**Ruggedness**

A study on system-to-system variability was conducted on many HPLC systems under comparable conditions and at different times. The test protocol called for the production and analysis of six samples. It is evident from comparing the two tests conducted on two distinct HPLC systems that the assay test process is unaffected by system variability. The RSD percentage was judged to be inside the mark. Refer to Tables 3 and 7.

**Robustness**

**The Impact of Flux Rate Variation:**

An investigation of the effects of flux rate variation was carried out. At flow rates of 1.0 and 1.2 milliliters per minute, the standard solution generated in accordance with the test protocol was introduced into the HPLC network. After testing, it was discovered that the machine's suitability parameters flowed between 1.0 and 1.2 milliliters per minute.
The retention durations for Vaborbactam and Meropenem were similar to those achieved using the 1.0 ml/min mobile method. Meropenem and Vaborbactam's tailing factor was found to be within acceptable bounds. in accordance with Table 8(i-ii).

**Specificity**

Normal and sample solutions had to be prepared for the test technique and then injected into the chromatographic equipment. In terms of retention time, the standard and sample chromatograms ought to be almost comparable. Both the Standard and the Sample had the same retention duration and identical chromatograms. As shown in picture 4(c). Meropenem has a Retention time of 4.707 minutes and Vaborbactam has a Retention time of 6.684 minutes.

**LOD and LOQ**

From linearity data, use the formula below to calculate the detection and quantitation limit.

$$\mathrm{LOD} = \frac{3.3\,\sigma}{\mathrm{S}}$$

σ = Normal reaction variance

S = Slope of Analyte calibration curve.

$$\mathrm{LOQ} = \frac{10\,\sigma}{\mathrm{S}}$$

σ = Normal reaction variance
S = Slope of Analyte calibration curve.

**Meropenem:**

The LOD and LOQ are determined from the linearity plot.:

$$\text{LOD} = \frac{3.3\,\sigma}{S} = \frac{3.3 \text{ X } 2796.31}{35296} = 0.261$$

$$\text{LOQ} = \frac{10\,\sigma}{S} = \frac{10 \text{ X } 2796.31}{35296} = 0.792$$

**Vaborbactam;**

$$\text{LOD} = \frac{3.3\,\sigma}{S} = \frac{3.3 \text{ X } 185.59}{5099} = 0.120$$

$$\text{LOQ} = \frac{10\,\sigma}{S} = \frac{10 \text{ X } 185.59}{5099} = 0.363$$

## DISCUSSION

Normal and sample preparation was necessary for the test technique. The analytical method was developed by the analysis of many parameters [13–16]. Meropenem's maximum absorbance was measured at 237 nm, but Vaborbactam's was at 275 nm. Peak purity was determined to be good, and the typical detection wavelength was 252 nm. The ideal peak area was obtained with an injection volume of 20 µL. The superior peak shape of the Inertsil C18 ODS column led to its selection. It was determined that the ambient temperature was adequate to preserve the drug solution's purity. The good peak area, adequate retention time, and high resolution led to the selection of a flow rate of 1.0 mL/min. The buffer's capacity to generate symmetrical peaks and high resolution led to its selection. The 80:20 methanol-to-buffer ratio produced the best results out of all the mobile phase ratios that were examined. The suggested approach then made use of this mobile phase ratio.

The results of the new analytical technique fell within the same 98.0–101.5% range as the guidelines, indicating that it was linear, accurate, and robut. It was shown that the procedure's and the device's precision were both dependable and within an acceptable range. For Meropenem and Vaborbactam, the detection limits were found to be 0.261 µg/mL and 0.120 µg/mL, respectively. Both medications' linearity was validated by the curve fitting and coefficient of correlation analysis. Both Meropenem and Vaborbactam showed linearity in the analytical method within the 20–80 ppm target concentration range. In every instance, the robustness and roughness tests were passed with outstanding relative standard deviations.

## CONCLUSION

The suggested approach, which is straightforward, precise, and cost-effective, and free from excipient interference, was successfully used for the quantification of Meropenem and Vaborbactam in the fixed dose formulation after being validated in accordance with ICH recommendations.

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**Table- 1(a): Meropenem system suitability data**

| Injection | RT | Peak Area | USP Plate count | USP Tailing |
|---|---|---|---|---|
| 1 | 4.712 | 1412455 | 10023.845712 | 1.158 |
| 2 | 4.707 | 1413987 | 10410.547812 | 1.121 |
| 3 | 4.705 | 1418948 | 10236.874214 | 1.141 |
| 4 | 4.706 | 1416555 | 10127.254178 | 1.134 |
| 5 | 4.707 | 1412589 | 10184.658952 | 1.145 |
| Mean | 4.704232 | 1414906 | 10336.825471 | 1.144 |
| SD | 0.00314 | 2796.31 | ------- | ------- |
| % RSD | 0.096627 | 0.197 | ------- | ------- |

**TABLE-1(b): Vaborbactam System Fitting Data**

| Injection | RT | Peak Area | USP Plate count | USP Tailing |
|---|---|---|---|---|
| 1 | 6.691 | 204258 | 8325.874512 | 1.036 |
| 2 | 6.684 | 204012 | 8384.547862 | 1.045 |
| 3 | 6.681 | 203902 | 8314.875424 | 1.056 |
| 4 | 6.680 | 204155 | 8372.784518 | 1.078 |
| 5 | 6.684 | 204365 | 8392.084512 | 1.033 |
| Mean | 6.686208 | 204138 | 8358.875421 | 1.047 |
| SD | 0.0.0507 | 185.59 | ------- | ------- |
| % RSD | 0.112138 | 0.090 | ------- | ------- |

**Table 2(a): Data of Linearity (Meropenem)**

| Concentration (ppm) | Average Area |
|---|---|
| 0 | 0 |
| 0 | 706445 |
| 30 | 1059486 |
| 40 | 1412450 |
| 50 | 1745687 |
| 60 | 2119450 |
| 70 | 2472451 |
| 80 | 2825606 |
| **Statistical Analysis** | |
| Slope | : 35296 |
| y-Intercept | : -1504 |
| Correlation Coefficient | : 0.999 |

**Table 2 (b): Details on linearity (Vaborbactam)**

| Concentration (ppm) | Average Area |
|---|---|
| 0 | 0 |
| 20 | 102056 |
| 30 | 153287 |
| 40 | 204256 |

| | |
|---|---|
| 50 | 248598 |
| 60 | 306482 |
| 70 | 357589 |
| 80 | 408513 |
| **Statistical Analysis** | |
| Slope | 5099 |
| y-Intercept | -489.1 |
| Correlation Coefficient | 0.999 |

**Table 3(i): Meropenem Information on Reliability (System Precise)**

| | Injection | Peak Areas of Meropenem | %Assay |
|---|---|---|---|
| **Concentration 40ppm** | 1 | 1413654 | 100.23 |
| | 2 | 1415782 | 100.38 |
| | 3 | 1412748 | 100.16 |
| | 4 | 1412320 | 100.13 |
| | 5 | 1414875 | 100.31 |
| **Statistical Analysis** | Mean | 1415501 | 100.36 |
| | SD | 1414146 | 100.26 |
| | % RSD | 1454.28 | 0.103 |

**Table 3(ii): Information on Vaborbactam Reliability (System Precise)**

| | Injection | Peak Areas of Vaborbactam | %Assay |
|---|---|---|---|
| **Concentration 40ppm** | 1 | 204511 | 100.67 |
| | 2 | 204687 | 100.76 |
| | 3 | 204015 | 100.43 |
| | 4 | 204194 | 100.52 |
| | 5 | 204906 | 100.86 |
| **Statistical Analysis** | Mean | 204735 | 100.78 |
| | SD | 204508 | 100.67 |
| | % RSD | 341.6513 | 0.167 |

**Table 4(ii): Vaborbactam repeatability data (precision technique)**

| | Injection | Peak Areas of Vaborbactam | %Assay |
|---|---|---|---|
| **Concentration 40ppm** | 1 | 204324 | 100.58 |
| | 2 | 204578 | 100.70 |
| | 3 | 204689 | 100.76 |
| | 4 | 204714 | 100.77 |
| | 5 | 204177 | 100.51 |
| | 6 | 204866 | 100.85 |
| **Statistical Analysis** | Mean | 204558 | 100.69 |
| | SD | 259.491 | 0.127 |
| | % RSD | 0.126 | 0.126 |

**Table 5(i): Data of Intermediate precision (Analyst 2) for Meropenem**

| | Injection | Peak Areas of Meropenem | %Assay |
|---|---|---|---|
| Concentration 40ppm | 1 | 1410056 | 99.97 |
| | 2 | 1405487 | 99.65 |
| | 3 | 1418789 | 100.59 |
| | 4 | 1414010 | 100.25 |
| | 5 | 1416545 | 100.43 |
| | 6 | 1415930 | 100.39 |
| Statistical Analysis | Mean | 1413469 | 100.21 |
| | SD | 4888.23 | 0.346 |
| | % RSD | 0.345 | 0.345 |

**Table 5(ii): Data of Intermediate precision (Analyst 2) for Vaborbactam**

| | Injection | Peak Areas of Vaborbactam | %Assay |
|---|---|---|---|
| Concentration 40ppm | 1 | 204148 | 100.49 |
| | 2 | 204025 | 100.43 |
| | 3 | 204298 | 100.57 |
| | 4 | 204151 | 100.49 |
| | 5 | 204286 | 100.56 |
| | 6 | 204568 | 100.70 |
| Statistical Analysis | Mean | 204246 | 100.54 |
| | SD | 187.32 | 0.091 |
| | % RSD | 0.091 | 0.091 |

**Table 6(i): Meropenem data with accuracy**

| Concentration % of spiked level | | Amount added (ppm) | Amount found (ppm) | % Recovery | Statistical Analysis of % Recovery | |
|---|---|---|---|---|---|---|
| 50% | Injection 1 | 20 | 20.05 | 100.28 | MEAN %RSD | 100.29 0.033 |
| | Injection 2 | 20 | 20.06 | 100.33 | | |
| | Injection 3 | 20 | 20.05 | 100.26 | | |
| 100% | Injection 1 | 40 | 40.05 | 100.14 | MEAN %RSD | 100.23 0.083 |
| | Injection 2 | 40 | 40.09 | 100.23 | | |
| | Injection 3 | 40 | 40.12 | 100.31 | | |
| 150% | Injection 1 | 60 | 60.09 | 100.01 | MEAN %RSD | 99.98 0.025 |
| | Injection 2 | 60 | 59.97 | 99.97 | | |
| | Injection 3 | 60 | 59.99 | 99.98 | | |

**Table 6(ii): Vaborbactam data with accuracy**

| Concentration % of spiked level | | Amount added (ppm) | Amount found (ppm) | % Recovery | Statistical Analysis of % Recovery | |
|---|---|---|---|---|---|---|
| 50% | Injection 1 | 20 | 20.17 | 100.88 | MEAN %RSD | 100.88 0.043 |
| | Injection 2 | 20 | 20.16 | 100.83 | | |
| | Injection 3 | 20 | 20.18 | 100.92 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 100% | Injection 1 | 40 | 40.24 | 100.62 | MEAN<br>%RSD | 100.62<br>0.084 |
| | Injection 2 | 40 | 40.21 | 100.54 | | |
| | Injection 3 | 40 | 40.28 | 100.71 | | |
| 150% | Injection 1 | 60 | 60.28 | 100.47 | MEAN<br>%RSD | 100.46<br>0.050 |
| | Injection 2 | 60 | 60.30 | 100.50 | | |
| | Injection 3 | 60 | 60.24 | 100.40 | | |

**Table 7(i): Data on System Variability (Meropenem) (System-2)**

| S.No | Peak area | Assay % of Meropenem |
|---|---|---|
| 1 | 1418465 | 100.57 |
| 2 | 1413568 | 100.22 |
| 3 | 1416875 | 100.46 |
| 4 | 1415536 | 100.36 |
| 5 | 1414750 | 100.31 |
| 6 | 1412842 | 100.17 |
| **Mean** | 1415339 | 100.35 |
| **%RSD** | 0.147 | 0.147 |

**Table 7(ii): Data on device variations (Vaborbactam) (System-2)**

| S.No | Peak area | Assay % of Vaborbactam |
|---|---|---|
| 1 | 204087 | 100.46 |
| 2 | 204298 | 100.57 |
| 3 | 204351 | 100.59 |
| 4 | 204624 | 100.73 |
| 5 | 204194 | 100.52 |
| 6 | 204550 | 100.69 |
| Mean | 204350 | 100.59 |
| %RSD | 0.100 | 0.100 |

**Table 8(i): There's proof that flux rate variability has an impact (Meropenem):**

| | Std Area | Tailing factor | | Std Area | Tailing factor | | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| **Flow 0.8 ml** | 1408784 | 1.011 | **Flow 1.0 ml** | 1412118 | 1.051 | **Flow 1.2 ml** | 1485978 | 1.089 |
| | 1408015 | 1.013 | | 1415487 | 1.087 | | 1483568 | 1.046 |
| | 1407009 | 1.011 | | 1412689 | 1.056 | | 1487521 | 1.084 |
| | 1407752 | 1.007 | | 1412594 | 1.039 | | 1485654 | 1.050 |
| | 1408329 | 1.016 | | 1418745 | 1.045 | | 1486895 | 1.064 |
| | 1405452 | 1.010 | | 1412290 | 1.035 | | 1482058 | 1.086 |
| **Avg** | 1407556 | 1.011 | **Avg** | 1413987 | 1.052 | **Avg** | 1485279 | 1.069 |
| **SD** | 1190.01 | 0.003 | **SD** | 2641.468 | 0.018 | **SD** | 2076.99 | 0.019 |
| **%RSD** | 0.0845 | | **%RSD** | 1.86 | | **%RSD** | 0.1398 | |

**Table 8(ii): Flow rate shift impact data (Vaborbactam)**

| | Std Area | Tailing factor | | Std Area | Tailing factor | | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| **Flow 0.8 ml** | 203145 | 1.078 | **Flow 1.0 ml** | 204235 | 1.097 | **Flow 1.2 ml** | 2022365 | 1.054 |
| | 203084 | 1.068 | | 204298 | 1.078 | | 2022984 | 1.036 |
| | 203198 | 1.058 | | 204659 | 1.089 | | 2022346 | 1.044 |
| | 203248 | 1.064 | | 204879 | 1.086 | | 2022684 | 1.024 |
| | 203265 | 1.044 | | 204098 | 1.080 | | 2022485 | 1.012 |
| | 203942 | 1.048 | | 204845 | 1.069 | | 2022895 | 1.032 |
| **Avg** | 203313 | 1.060 | **Avg** | 204502 | 1.083 | **Avg** | 2022626 | 1.033 |
| **SD** | 314.9785 | 0.015 | **SD** | 334.8275 | 0.009 | **SD** | 272.1358 | 0.014 |
| **%RSD** | **0.154** | | **%RSD** | **0.163** | | **%RSD** | **0.013** | |

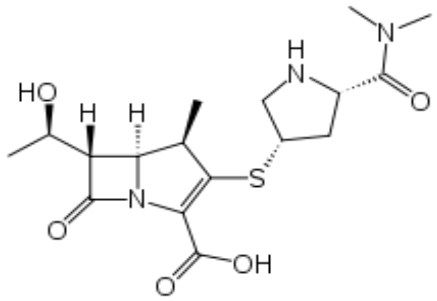


**Figure 1: Structure of Meropenem**

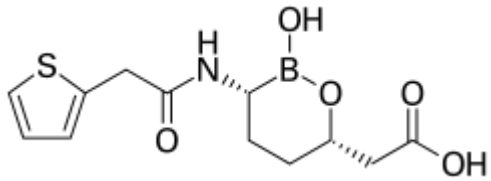


**Figure 2: Structure of Vaborbactam**

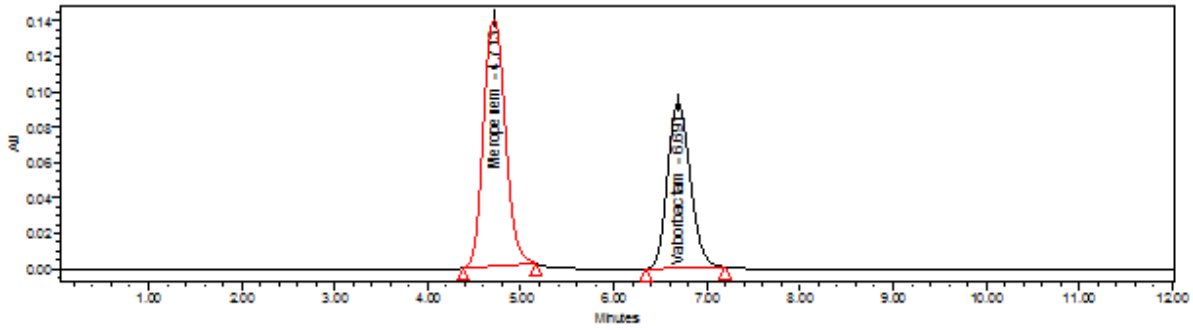


**Fig 3: Chromatography in its traditional form**

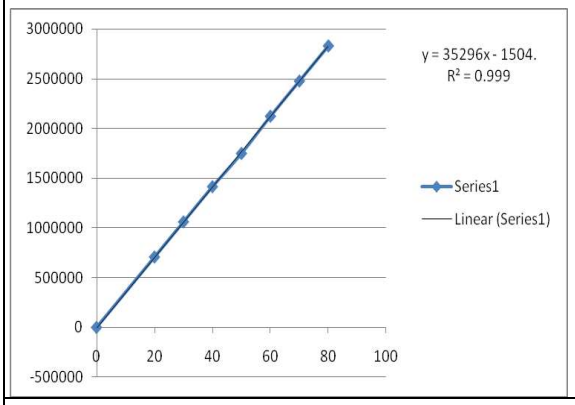


**Fig: 4(a) Meropenem's Linearity Plot**

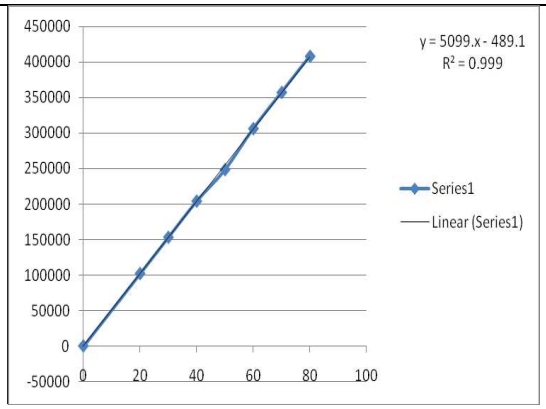


**Fig: 4(b) Plot of Vaborbactam**

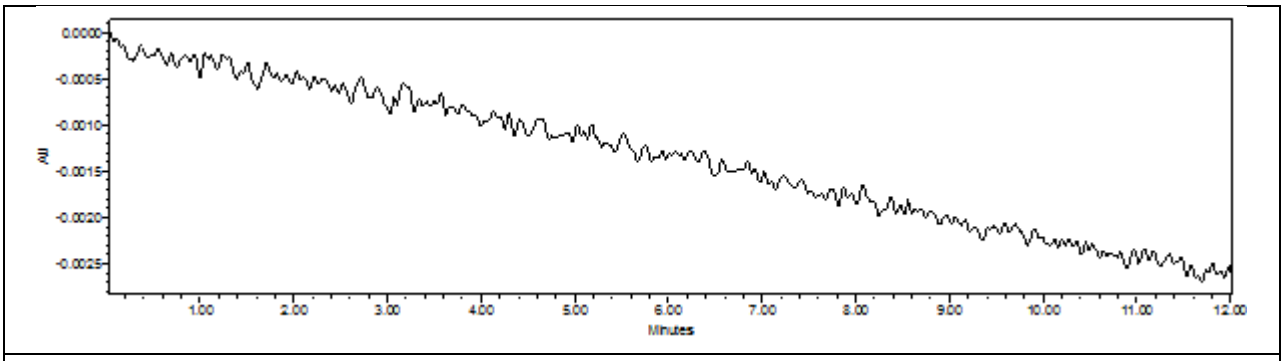


**Fig 4(c): Blank Chromatograph**

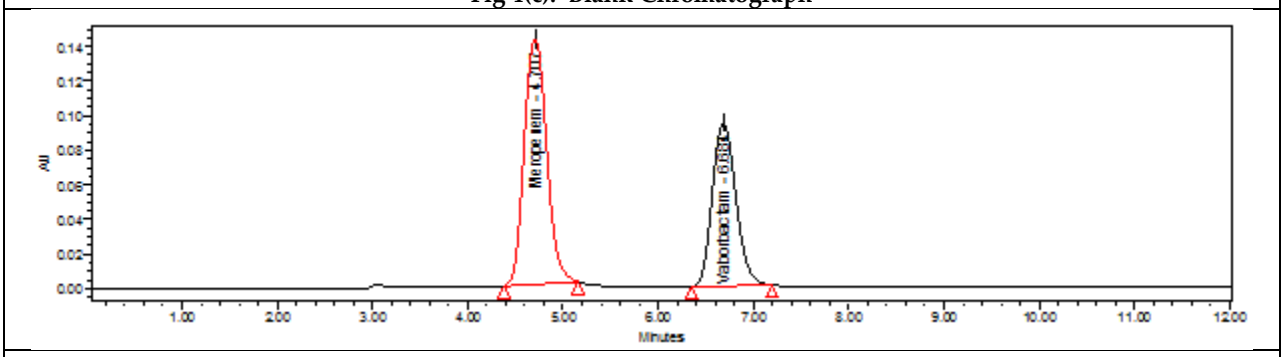


**Fig 5: Chromatogram Standard**

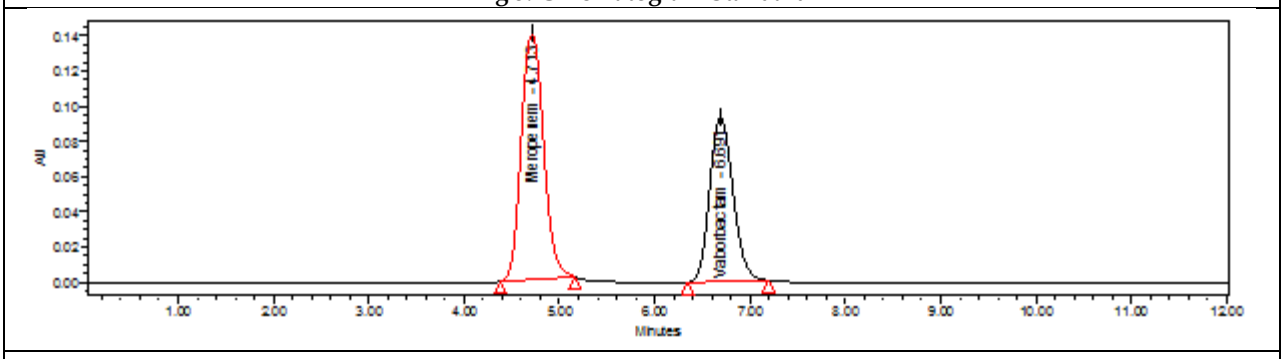


**Fig 6: Chromatogram Sample**

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**RESEARCH ARTICLE**

# Innovative ML Methods for Automatic Blood Cell Images Analysis and Labeling: Improving Medical Imaging Diagnostic Precision with Extracting Features and Classification Approaches

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## ABSTRACT

Advanced machine learning methods to automatically analyze and categorize images of blood cells with the goal of enhancing the precision of medical imaging diagnosis. The work focuses on identifying important structural characteristics in red and white blood cells by utilizing extraction of characteristics and segmentation methods. Deep learning models are among the sophisticated methods used to improve picture segmentation and classification procedures. The suggested techniques have the ability to effectively identify blood illnesses such as leukemia, anemia, and infections, resulting in quicker and more accurate diagnosis. According to experimental findings, these automated techniques perform better than conventional techniques with respect to overall precision, memory, and overall accuracy, which qualifies them for use in clinical settings.

**Keywords :** Medical Imaging, Feature Extraction, Machine Learning.

# INTRODUCTION

Early disease diagnosis, treatment, and detection depend heavily on medical imaging. Specifically, the diagnosis of hematological illnesses such as anemia, leukemia, and infections depends heavily on the study of blood cell images[1,2]. The conventional procedure has been for pathologists to manually examine blood smears; however, this procedure is laborious, subjective, highly highly susceptible to accidental mistakes. Because imaging techniques and computerisation have made analyses faster and more precise, diagnostic procedures have been greatly enhanced[3,4,5]. But even with these advancements in technology, issues with accuracy and scalability still exist[6]. Because machine learning (ML) approaches can automate and improve the reliability of blood vessels characterisation and overall diagnostic processes, there has been an increase in interest in these techniques as a result[7]. Medical imaging diagnostic procedures can now be automated with great power thanks to machine learning, especially when it comes to picture processing[8]. Machine learning (ML) methods in blood vessel analysis can be utilized to spot complex patterns and anomalies that human observers might overlook[9]. Large datasets of labeled blood cell images are used by these mathematical models to help them learn to distinguish between diseased and normal cells without an exceptionally high level of accuracy[10,11]. Additionally, predictive models are very effective at handling large amounts of data in a short amount of time, which makes them ideal for regular diagnostic tasks[12]. By lowering error rates and enhancing patient outcomes, such abilities have the capacity to revolutionize diagnostic operations[13]. In medical image analysis, including blood cell categorisation, obtaining features is an essential first step[14]. In order to use a picture as an input for machine learning algorithms, it is necessary to recognise and isolate important features from the image. Shape, size, texture, including color are examples of traits that can be found in tissue analysis[15]. These aspects are crucial for differentiating between different blood cell types and identifying pathological abnormalities. Models created using machine learning can focus on the most important details of a picture with the help of efficient feature extraction, which increases classification accuracy[16]. The technique of feature extraction has been significantly improved by recent developments in deep learning, enabling more sophisticated analysis of complicated images with no requiring a lot of manual preliminary processing. In the computerized examination of medical images, segmentation is yet another crucial step[17]. Segmentation algorithms are employed in blood cell imaging to distinguish individual cells between background and additionally overlapping cells, an important step towards precise categorisation[18]. The presence of artifacts in the images and the variety in cell morphology can make this process difficult. Such complications are beyond the capabilities provided by conventional segmentation techniques like thresholding along with edge detection[19]. More accurate blood cell picture segmentation has been demonstrated using contemporary machine learning techniques, especially convolutional neural networks (CNNs). More precise and trustworthy detection of aberrant neurons in clinical settings is now possible because of these developments[20].

# METHODOLOGY

By incorporating modern machine learning methods within blood cell analysis, evaluation accuracy could be greatly increased. These algorithms can increase uniformity among diagnoses and lessen the inherent disparities in assessments by humans by simplifying the identification process. Furthermore, the ability to continually develop and refresh machine learning models with fresh data enables them to keep up with the rapidly advancing field of medicine. This flexibility is particularly crucial for diagnosing uncommon or unusual cases, when human experience may be scarce. Research has demonstrated that machine learning techniques can perform better for classifying blood cells than conventional techniques that are manual in regard to acceleration, awareness, and precision.

**Activity Diagram for the Blood Cell Image Processing Workflow**

This illustrates the steps involved in making decisions and deciding the categorisation outcome while analyzing blood cell images Because deep learning, a kind of machine learning, is capable of distinguishing hierarchical characteristics from unprocessed data, it has become increasingly popular in the field of medical picture analysis. Deep learning models, with the value recurrent neural networks (RNNs) and CNNs, have shown impressive results

in blood cell categorisation. These models enable more precise distinction between healthy and sick cells by capturing both the regional and global characteristics of blood cells. Deep learning models are especially useful for difficult and high-dimensional tasks including blood cell analysis because they can independently retrieve pertinent features from huge datasets, in contrast to typical statistical techniques that depend on manually generated features. Partitioning an image into recognizable portions that correspond to distinct structures, like particular blood cells, is a crucial step in the analysis of blood cells. This procedure is essential for separating individual cells within their surroundings and guaranteeing that every cell gets examined separately. Precise segmentation is crucial for the later phases of features being extracted and categorized because improperly divided images can provide machine learning models with inaccurate or insufficient data. Machine learning-driven approaches to segmentation have made tremendous progress in handling noisy and elaborate medical pictures, especially when cells have irregular shapes or overlap. Machine learning approaches are additionally being used to predict how a disease would progress and how the proposed therapy will be implemented based on images of blood cells. In diseases such as leukemia for example, where early identification and surveillance are crucial, machine learning algorithms assist in detecting subtle modifications to red blood cells in existence composition that may indicate the onset or progression of the disease. By providing real-time analysis and ongoing monitoring, these innovations can assist healthcare providers in making more informed decisions regarding treatment regimens and patient care. The technique of machine learning possesses the ability to completely transform the discipline of hematology by offering faster diagnosis times along with more individualized and accurate medication options .

## RESULTS ANALYSIS

The below table represents the Predicted blood cells
Healthy vs Abnormal
Through replicating cells with white circles against a dark backdrop, the generate_synthetic_data utility produces artificial plasma cell images.
At random, labels are allocated as aberrant (0) or healthy (1).

## CONCLUSION

The continuous improvement of predictive algorithms and their incorporation into clinical workflows hold the key to the next generation of automation blood cell analysis. New methods that have the potential to overcome existing constraints include transfer learning, which enables models to utilize data collected from related tasks, and generative adversarial networks (GANs), and this can enhance small datasets. Furthermore, the creation of more comprehensible models will boost doctors' confidence in diagnosis based on machine learning. Those methods will probably develop into an essential tool in hematology as they get more advanced, allowing for quicker, more precise, and easier access to diagnosis for a variety of life-threatening illnesses, ultimately leading to better clinical results and care.

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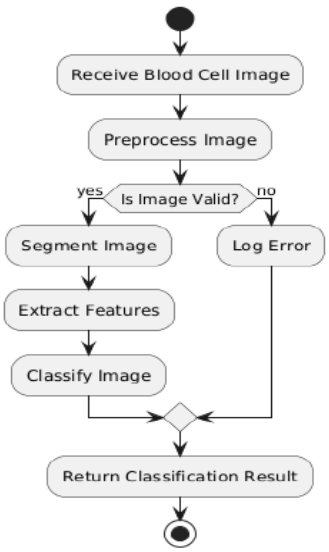

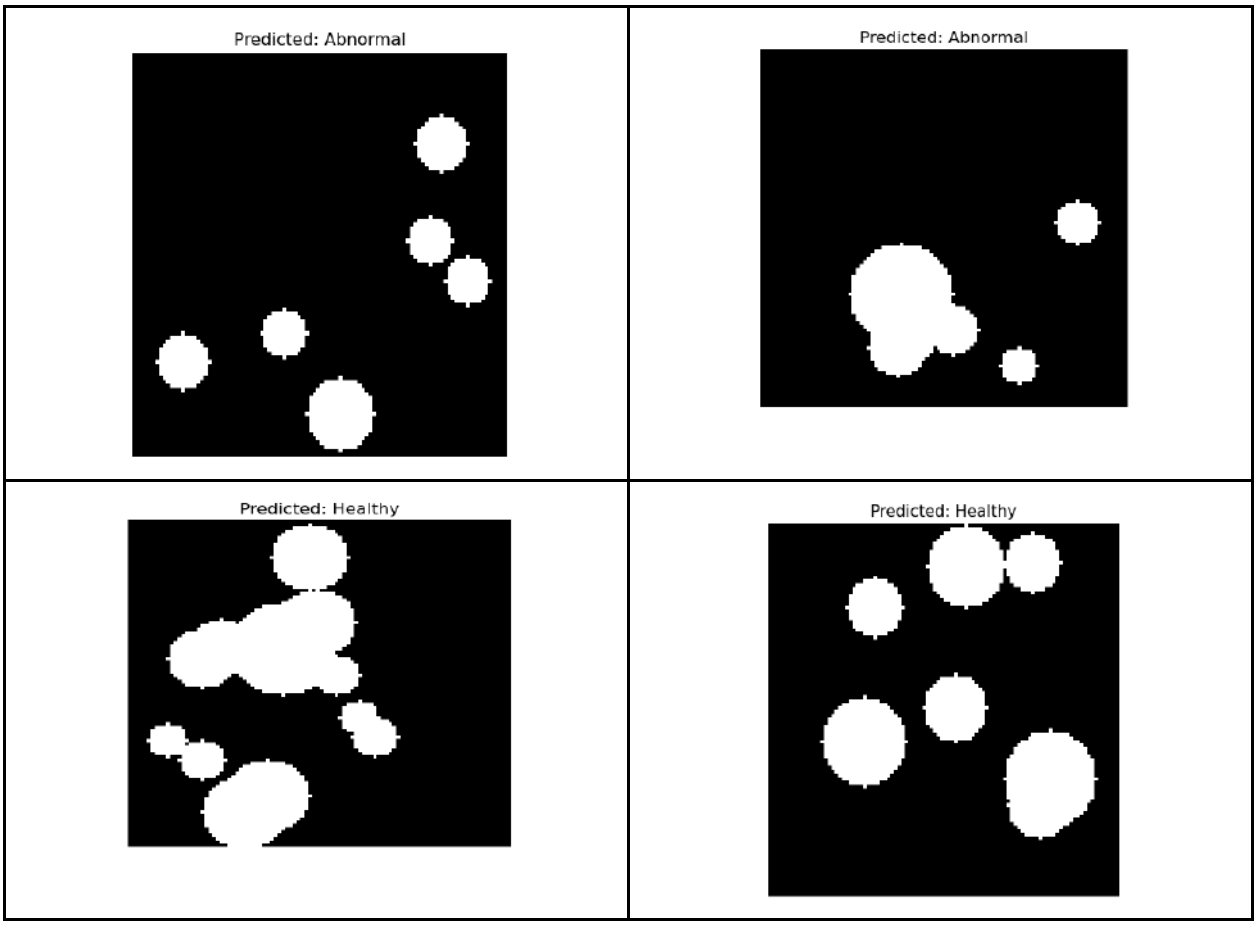


## Classification Report

| | precision | recall | f1-score | support |
|---|---|---|---|---|
| 0 | 0.4296875 | 0.6111111111111112 | 0.5045871559633027 | 90.0 |
| 1 | 0.5138888888888888 | 0.33636363636363636 | 0.40659340659340654 | 110.0 |
| accuracy | 0.46 | 0.46 | 0.46 | 0.46 |
| macro avg | 0.4717881944444444 | 0.4737373737373738 | 0.45559028127835466 | 200.0 |
| weighted avg | 0.4759982638888889 | 0.46 | 0.45069059380985976 | 200.0 |

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**RESEARCH ARTICLE**

# Formulation of Geomatrix Extended Release Tablets of Metformin HCl using CO Processed Gums

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## ABSTRACT

Metformin is a water-soluble drug and it acts primarily by reducing hepatic glucose production by enhancing insulin sensitivity in peripheral tissues their by lowering the blood glucose levels.In this research initially co processed gums were prepared using Xanthin gum and Acacia gum. The geomatrix tablet contains active middle layer and two barrier layers to retard the drug release. The active middle layer in development of geo matrix tablets was consist of metformin and coprocessed gums. Tablets were prepared by the direct compression by sandwich active middle layer with two barrier layers. These tablets were evaluated for various pre, post compression parameters and in vitro dissolution studies for 24 hours. FTIR studies proved that there was a good compatibility between drug and excipients. All the geo matrix tablets were showed significant retardation in drug release and geo matrix tablet formulation CM5showed maximum drug release at end of 24 hours.

**Keywords:** metformin, extended release,geo matrix tablets,coprocessed gums.

## INTRODUCTION

Pharmaceutical excipients are defined as the substances other than the API which has been appropriately evaluated for safety and are intentionally included in a drug delivery system [1]. The International Pharmaceutical Excipients Council (2009) defines excipients as the substances which present in a finished pharmaceutical dosage form other than the active drug substance [2]. Excipient can be classified into four categories generally: single entity excipient, a physical blend of multiple excipients, new chemical entity excipient and co-processed excipient [3]. An excipient that has undergone co-processing is a combination of two or more 84418 possible84418 or non-compendial excipients with the goal of physically changing their properties without altering chemical properties.[4] There is no single-component excipient 84418 possible all the requisite performance to allow an active pharmaceutical ingredient to be formulated into a specific dosage form. Co-processed excipient has received much more attention in the formulation development of various dosage forms, specially for tablet preparation by direct compression method.

The pharmaceutical industry first started to use co-processed excipients from the late 1980s, with early examples including co-processed microcrystalline cellulose and calcium carbonate, introduced in 1988, MEGGLE's co-processed cellulose and lactose (1990), and co-processed glucomannan and galactomannan (1996). The geo-matrix multilayer tablet technology was developed by conte and co-workers for constant drug release [5]. The technology incudes triple – layered and bi-layered tablets. The triple-layered tablet which is exemplified consists of an active core which is a hydrophilic matrix layer and two polymeric barriers layers on either side that are hydrophobic or semi permeable [6]. The Bi-layered tablet consist of the drug layer and one barrier layer . The barrier layer modifies the swelling rate of the active core and reduces the surface area available for diffusion of drug [7,8]. Zero-order drug release can be achieved with the Geo-matrix system [9]. However release is limited to one drug**.** Metformin is a BCS-I drug and Soluble in water, slightly soluble in alcohol, practically insoluble in acetone and methylene chloride.It acts primarily by reducing hepatic glucose production by enhancing insulin sensitivity in peripheral tissues their by lowering the blood glucose levels[10,11].

## EXPERIMENTAL METHODOLOGY

**Preparation of co – processed gums**

Xanthan gum and acacia gum, at concentrations of 3% each, were dispersed in distilled water. The xanthan gum and acacia gum were mixed at varying ratios ( 1:2, 2:1, 1:3, and 3:1) and homogenized with a rotation of 3000 rpm for 30 min to obtain a homogeneous mass. The mass was then dried with a hot air drier at 80±5°C. The obtained layer or flakes were milled and sieved using a 60 mesh. Functional characterizations of the mass were then performed.The process of preparation is shown in Figure-1 .

**Drug – Excipient Compatibility Studies by FTIR:**

Compatibility study was performed by making ready compatibility blends at completely ratios of various excipients with the drug, supportive tentative average weight. These blend were stored at accelerated condition of 40ºC/75%RH. Control samples were stored at 40ºC. the ratio of the drug to excipient varies from 1:1 to 1:10 depending on the purpose of use, and the samples were kept in double lined poly- bags. These samples were evaluated for any change in the physical characteristics with reference to its controlled sample stored at 40ºC for a period of 15 days. In the present study, the potassium bromide disc (pellet) method was employed. Chemical stability was confirmed by IR spectrometry.

**Preparation of middle active layer Using co processed gums**

The middle active layer was prepared by direct compression method using co processed gums using xanthan and acacia gums. The formulation was prepared according to the formulation table given in Table-1. These materials were screened through ≠60 and mixed together in motor by using pestle. Final mixtures were compressed by using

11mm diameter flat punches on a eight- station rotary tablet press. The prepared tablets were subjected to dissolution studies.

**Preparation of upper and lower layers**

The barrier layers were formulated employing hydrophilic swellable polymer HPMC K100M and Ethyl Cellulose. The procedure tried to make the compacts was via direct compressions. For the first procedure the both polymers were mixed in mortar and lubricated with magnesium stearate.

**Formulation of Geo matrix (Tri-layer) tablets**

The powder mixtures required for active and barrier layers were weighed accurately and thoroughly mixed using mortar and pestle for about 20 minutes. Initially, the volume of die cavity; (12mm, round) was adjusted equivalence to the weight of tri-layered matrix tablets (900mg). Then the pre-weighed amount of powder equivalent to bottom layer (25mg) was taken and placed in the die cavity and slightly compressed for uniform spreading. The upper punch was lifted and 850mg of the drug containing middle active layer was placed over the bottom layer in the die cavity and again slightly compressed. The remaining volume of the die cavity was filled with pre-weighed (25mg) amount of powder equivalent to top layer and compressed with the full force of compression on rotary tablets press to obtain tri-layered tablets. The schematic diagram of geomatrix tablet is shown in Figure-2.

**Evaluation of Pre compression Parameters:**

All the formulations precompression parameters like Bulk density,Tapped density, Compressibility index and angle of repose were determined according to the official methods.

**Evaluation of Post compression Parameters:**

All the formulations post compression parameters like hardness,Friability,average weight and drug content were determined according to the pharmacopoeial procedures.

***In vitro* dissolution studies of Extended- Release Geo-Matrix Tablets**

Drug release from the of geo matrix tablets were studied using USP I basket dissolution apparatus, in 900 mL of pH 6.8 Phosphate buffer for 18 hours at 37±0.5ºC at 75 rpm. 5 mL of dissolution medium was withdrawn using a syringe fitted with 0.45 µm pre filter at regular time intervals and the same volume of (37±0.5ºC) fresh dissolution medium maintained at 37±0.5ºC was replaced. The samples were diluted wherever necessary and analyzed at appropriate wavelengths for Metformin spectrophotometrically. The cumulative percent of drug released at different time intervals was calculated. All the dissolution studies were performed 6 times and average values were reported.

**Drug release kinetics and mechanism**

The rate and mechanism of drug release play an important role in assessing the quality of the dosage form and also for achieving the desired time of drug action in the body for controlled release dosage forms. The drug release kinetics was evaluated by fitting the data to zero and first order. Mechanism of drug release was evaluated by fitting the data to Higuchi diffusion model, erosion model and further characterized by using Korsmeyer-Peppas equation.

## RESULTS AND DISCUSSION

**Functional group analysis**

The purpose of the functional group analysis was to ascertain whether a chemical reaction occurred between each of the functional groups of the two excipients. Xanthan gum and acacia gum only interact or mix physically. No new functional groups presented in the spectrum (Fig. 1), indicating that these interactions do not involve chemical changes. The Fourier-transform infrared spectroscopy spectrum of xanthan gum, at wavenumber 1780.8 cm−1 showed a sharp peak, suggesting the presence of −C=O carboxylic bonds. This is reinforced by the presence of wide peaks with strong intensities in the range of wavenumbers 2500–3300 cm−1, indicating the presence of the −OH carboxylate group.

**Drug – Excipient Compatibility Studies :**Overall there was no alteration in peaks of Metformin pure drug (Figure 3) and optimized formulation (Figure 10), suggesting that there was no interaction between drug & excipients. FTIR spectrum of pure drug and other polymers is shown in (Figure 4). There is no additional peaks appeared or disappeared hence no significant changes in peaks of optimized formulation was observed when compared to pure drug indicating absence of any interaction

**Precompression Parameters**

The bulk density (g/ml) & tapped density of powdered blend of all the formulations is found to be in therange of 0.61 to 0.66 & 0.7 to 0.75 respectively. All formulations are within the pharmacopeial limits.

The CI & angle of repose of powdered blend of all the formulations is found to be in the range of 10.81 to 13.89 & 31.16 to 34.89 respectively.All formulations are within the pharmacopeial limits.

**Post Compression Parameters**

The hardness of all formulations was measured in kg/cm$^2$. Hardness of all formulations was in the range of 4.5±1.02 to 6.1 kg/cm$^2$ and friability values of none of the formulations exceeded 1% the results of friability indicate that the tablets were mechanically stable.

The weights & Drug content of the tablets were between 849.02 ±2.789 to 852.86±2.988 mg & 97.5±0.50 to 102.7±0.21 respectively.All the post compression parameters given in Table-2

***In vitro* dissolution studies of extended release metformin tablets:**

The *In vitro* drug profile of metformin from different formulations was carried out in phosphate buffer pH 6.8, and the results are depicted in Figure-5 & 6.The geo matrix tablets extended the drug release up to 24 h. Among all the formulations AM5 & BM5 failed to retard drug release upto 24 hours because of low polymer content in coprocessed gums. Formulations CM5 & DM5 retarded drug release upto 24hours. because of high polymer content in coprocessed gums. The highest drug release was found in the formulation CM5 i.e 99.98±0.05% within 24 h. CM5 was found to be optimized formulation based on the dissolution and other evaluation parameters. In the present study drug release mechanism is best fitting to zero order and Higuchi model because regression coefficient was seen closest to 1 in these models which conforms diffusion assisted mechanism of release. The correlation coefficient values are given in Table-3.

## CONCLUSION

Based on the evaluation parameters, drug dissolution profile and release drug kinetics CM5 was found to be optimized formulation. The drug release from CM5 was found to fit Zero order of concentration independent and best fitted to Higuchi's model confirming to be diffusion assisted mechanism. The developed drug delivery systems showed prolonged and complete drug release rates over a period of 24 h. These results also demonstrated the suitability of geo matrix (three-layered) tablet formulation of metformin to provide controlled release for prolonged period of time and improved linearity for metformin in the effective management of diabetes with patient compliance. It was concluded that geo matrix tablets of Metformin could be successfully prepared by direct compression technique using different coprocessed excipient combination. Metformin tablets were prepared by direct compression and consist of middle active layer with different co processed gum and upper and lower layers were prepared with HPMCK100M, ethyl cellulose,Talc and Magnesium stearate.

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**Table-1.Formulation Table**

| INGREDIENTS | FORMULATION CODE | | | |
|---|---|---|---|---|
| | AM5 | BM5 | CM5 | DM5 |
| **MIDDLE LAYER** | | | | |
| Metformin HCl | 500 | 500 | 500 | 500 |
| Co-XG-AG 1:2 | 300 | - | - | - |
| Co-XG-AG 2:1 | - | 300 | - | - |
| Co-XG-AG 3:1 | - | - | 300 | - |
| Co-XG-AG 1:3 | - | - | - | 300 |
| Talc | 8 | 8 | 8 | 8 |
| Magnesium stearate | 8 | 8 | 8 | 8 |
| Micro crystalline cellulose | 34 | 34 | 34 | 34 |
| **TOP LAYER** | | | | |
| Ethyl Cellulose | 10 | 10 | 10 | 10 |
| HPMC K100M | 10 | 10 | 10 | 10 |
| Talc | 1 | 1 | 1 | 1 |
| Magnesium stearate | 1 | 1 | 1 | 1 |

| BOTTOM LAYER | | | | |
|---|---|---|---|---|
| Ethyl Cellulose | 10 | 10 | 10 | 10 |
| HPMC K100M | 10 | 10 | 10 | 10 |
| Talc | 1 | 1 | 1 | 1 |
| Magnesium stearate | 1 | 1 | 1 | 1 |
| **Total Weight of Tablet** | 900 | 900 | 900 | 900 |

**Table-2.Post compression parameters**

| Formulationcode | Hardness | Friability | Average weight | Drug contentd |
|---|---|---|---|---|
| | (Kg/cm2) | (%) | of tablet sc (mg) | (%) |
| **AM5** | 4.5±1.02 | 0.48 | 901.26±2.268 | 99.5±0.60 |
| **BM5** | 4.1±0.98 | 0.32 | 901.02±2.789 | 101.7±0.67 |
| **CM5** | 4.2±1.23 | 0.29 | 900.86±2.186 | 99.±0.97 |
| **DM5** | 4.1±1.25 | 0.25 | 901.02±2.868 | 101.2±1.54 |

**Table-3. Correlation coefficient values of different kinetic models of drug release of all formulations**

| Formualtion | Zero order | First order | Higuchi model | Korsmeyers model | n value |
|---|---|---|---|---|---|
| AM5 | 0.9924 | 0.9495 | 0.9905 | 0.9466 | 0.989 |
| BM5 | 0.9939 | 0.9488 | 0.991 | 0.9416 | 0.994 |
| CM5 | 0.9936 | 0.9304 | 0.9909 | 0.9435 | 0.9923 |
| DM5 | 0.9939 | 0.8714 | 0.991 | 0.9416 | 0.994 |

**Figure-1.Different steps involved in preparation of co processed gums**

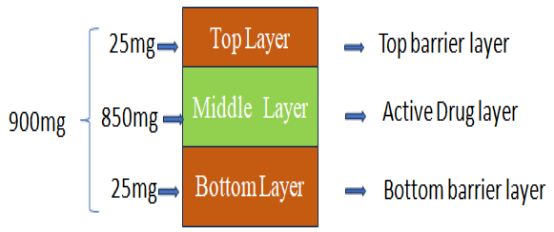


**Figure-2. Schematic diagram of geo matrix tablet**

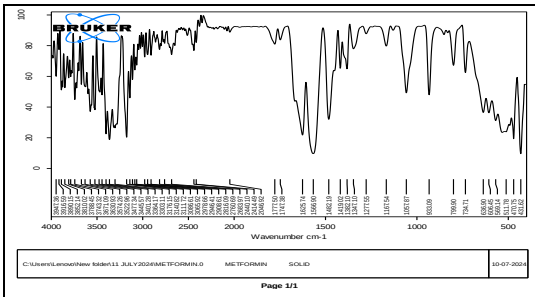


**Figure-3.FT-IR spectrum of pure metformin**

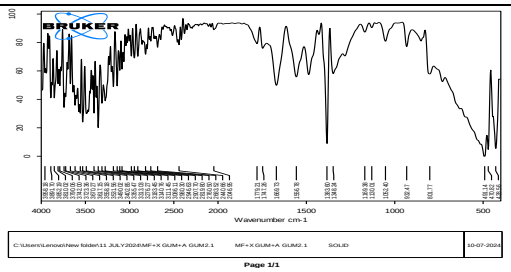


**Figure-4.FT-IR spectrum of pure metformin with co processed gum**

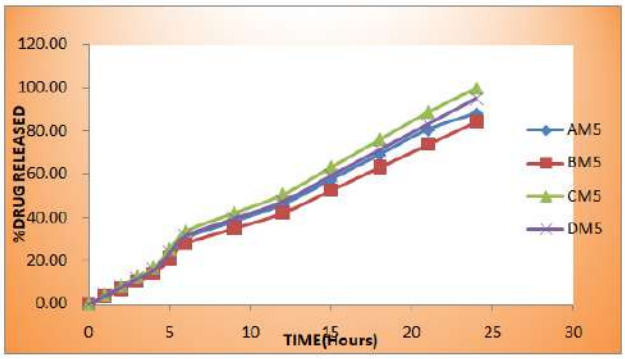


**Figure 5 Dissolution Profile of Geomatrix tablets**

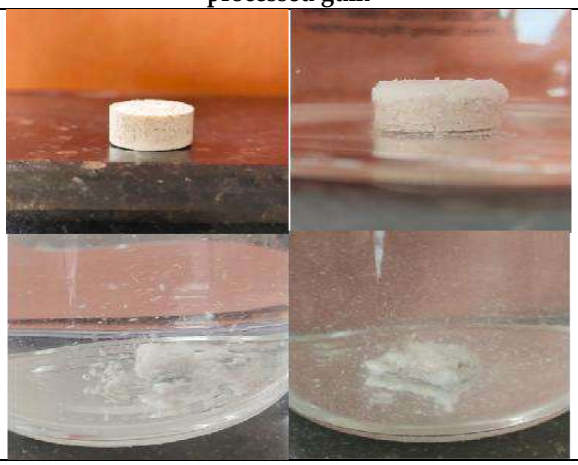

**Figure 6. Dissolution study of geomatrix tablet at different time intervals**